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EPIDEMIOLOGICAL STUDIES ON BLUETONGUE VIRUS INFECTION IN WEST JAVA, INDONESIA

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Abstract

EPIDEMIOLOGICAL STUDIES ON BLUETONGUE VIRUS INFECTION IN WEST JAVA, INDONESIA.

In monitoring of sentinel cattle in West Java, seroconversions to orbiviruses occurred mostly at the end of the wet season. A low altitude site gave more reactors than did a high altitude site. Due to perceived inefficiencies of the agar gel immunodiffusion (AGID) test, a competitive ELISA (C-ELISA) was applied and the results compared with the AGID test results. C-ELISA detected antibodies at an earlier stage of infection than did the AGID test. Not all sera reacting in the AGID test reacted in C-ELISA, suggesting that the C-ELISA is more specific in detecting bluetongue virus (BTV) antibodies than the AGID. However, as the infection status of most field sera was not known, this could not be confirmed conclusively from the available data. A comparison of isolation methods indicated that isolates were obtained more frequently if samples were passaged in embryonated eggs before blind passage in *Aedes albopictus* cells followed by passage in BHK-21 cells. Six BTV serotypes, 1,7,9,12,20, 21 and 23 were identified and confirmed from apparently healthy sentinel cattle blood at low altitudes; BTV serotype 21 was also isolated from a pool of the Avaritia sub-genus of the Culicoides spp which contained 227 *C. fulvus* and 20 *C. orientalis*.

1. INTRODUCTION

Twenty four bluetongue virus (BTV) serotypes have so far been identified. The viruses infect ruminants, but clinical signs have been reported mostly in sheep which show high fever, facial oedema, hyperaemia, ulceration of mucous membranes and inflammation of the coronary bands [1].

In Indonesia, clinical signs have not been reported in local ruminants. However, in serological surveys more than 60% of large ruminants and less than 30% of small ruminants were reactors in the agar gel immunodiffusion (AGID) test [2, 3]. Serum neutralization tests against 5 BTV serotypes and 1 type of the related epizootic haemorrhagic disease of deer virus (EHDV) indicated that Indonesian ruminants had antibodies against these orbiviruses with varying prevalences [4].

Based on these preliminary results sentinel herds were established in West Java at high and low altitudes to gain more information on the seasonality of BTV infection and to obtain isolates from weekly blood collections [5]. Earlier work yielded two serotypes of BTV, 7 and 9, from blood of sentinel cattle in the low altitude area [6]. Since then, further isolations from cattle blood have been conducted and the results are presented here.

Although BTV is an arthropod-born virus spread by *Culicoides* spp., vector studies on BTV have not yet been reported in detail. Isolation from insects has been attempted and the results are reported here.

2. MATERIALS AND METHODS

2.1. Viruses

BTV 1 (CSIRO 156) and BTV 20 (CSIRO 19) were used as antigens for the agar gel immunodiffusion test. BTV 1 and 20 were supplied by T.D. St.George of the Division of Tropical Animal Production, Brisbane, Australia.

2.2. Sentinel herds

Sentinel dairy cattle were established at two sites in West Java, Depok as a low altitude area (70 m) with 3,500 mm annual rainfall and Cisarua as a high altitude area (1300 m) with 3,500 mm annual rainfall [5]. Sentinel cattle were monitored from June 1986. Each group of 10-20 cattle was used for one year and changed with a new group in the same area. Sera were collected monthly for serological testing to gain information on seasonality of BTV infections and heparinized blood was collected weekly for viral isolation. Sentinel goats and sheep were also established in 1987 at a high altitude area (700 m, Cikidang), and bled at monthly intervals for 15 months. A second group of sentinel goats and sheep (8 goats and 4 sheep) was established in the same area in 1991 and bled at monthly intervals for 11 months.

2.3. Serological testing

2.3.1. Competitive ELISA (C-ELISA)

The C-ELISA test was performed on all sera from sentinel cattle. Antigen derived from BTV serotype 1 and monoclonal antibody 3-17-A3 was supplied by Dr. J. Anderson of the Pirbright Laboratories, UK. The method used was that described by Anderson [7].

2.3.2. Agar gel immunodiffusion (AGID) test

The AGID test was carried out as described by Della-Porta *et al* [8] and Sendow [3]. Antigen used in this test was prepared from BTV 20 for the samples from 1986 to 1989 and from BTV 1 for the samples from 1990 to 1991.

2.4. Insect collection

Insects were collected since February 1991 at both sentinel cattle sites using Pirbright miniature light traps [9]. Insects were identified to gain more information of *Culicoides* spp. present in the study areas. Identified *Culicoides* were also used for viral isolation.

2.5. Viral isolation

Heparinized blood from weekly collections from sentinel animals and pools of *Culicoides* spp. were investigated. Blood samples collected between 1986 and 1990 were inoculated directly into BHK-21 cell cultures. Blood samples collected between 1990 and 1991 were inoculated into 11 day old embryonated chicken eggs (ECE) before passaging to *Aedes albopictus* (C6/36) and BHK-21 cells. The method was described in Sendow *et al.* [6].

2.6. Identification of viral isolates

Isolates showing CPE in cell culture were identified to the bluetongue serogroup using immunodotblotting (IDB) [10]. The AGID test [8] or the fluorescent antibody technique (FAT) [11] were also used previously in detecting orbiviral group antigens. Hyperimmune rabbit antisera against BTV1 virus was supplied by Dr. A.J. Della-Porta, AAHL, Australia for the AGID and FAT tests. A monoclonal antibody specific to BTV viral protein VP7 and supplied from AAHL through Drs I. Polkinghorne and G. Burgess, Australia was used in the IDB test. Isolates containing BTV antigen were marked by a brown colored dot on nitrocellulose paper in the test where the immunoperoxidase conjugate detected the binding of the monoclonal antibody [12].

3. RESULTS

At the start of these observations, antibody was detected in calves at the low altitude area using the AGID (Fig. 1). These animals were less than 3 months old, hence maternal antibody may have been involved. During the 3 years of study (1987-1990), seroconversions occurred mostly in February/March at the low altitude site and during May-June at the high altitude site (Fig. 2). A higher prevalence of infection was recorded at the low altitude site than in the high altitude area using the AGID test. No seroconversions were recorded in 1990/1991.

Sentinel goats and sheep established at a high altitude site did not seroconvert in either the AGID or C-ELISA.

The AGID and C-ELISA results from the sentinel cattle are presented in Tables I and II. Thirty three (33) sera were positive in the AGID but negative in the C-ELISA using BTV 20 as AGID antigen. This may be related to the specificity of the test. Seventy two (72) sera were negative in the AGID but positive in C-ELISA. Similar results were obtained using BTV1 as AGID antigen as shown in Table II.

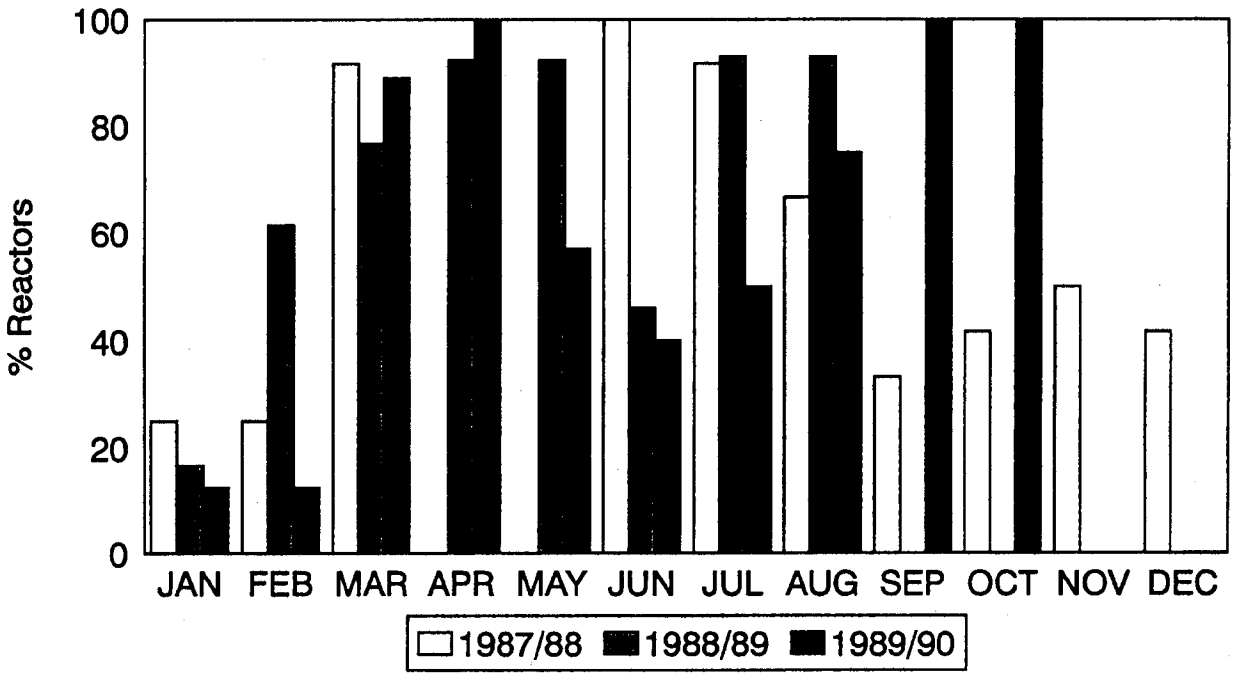

FIG. 1. Percentage of reactors to BTV in sentinel cattle at Depok, a low altitude area (June 1987 to 1990).

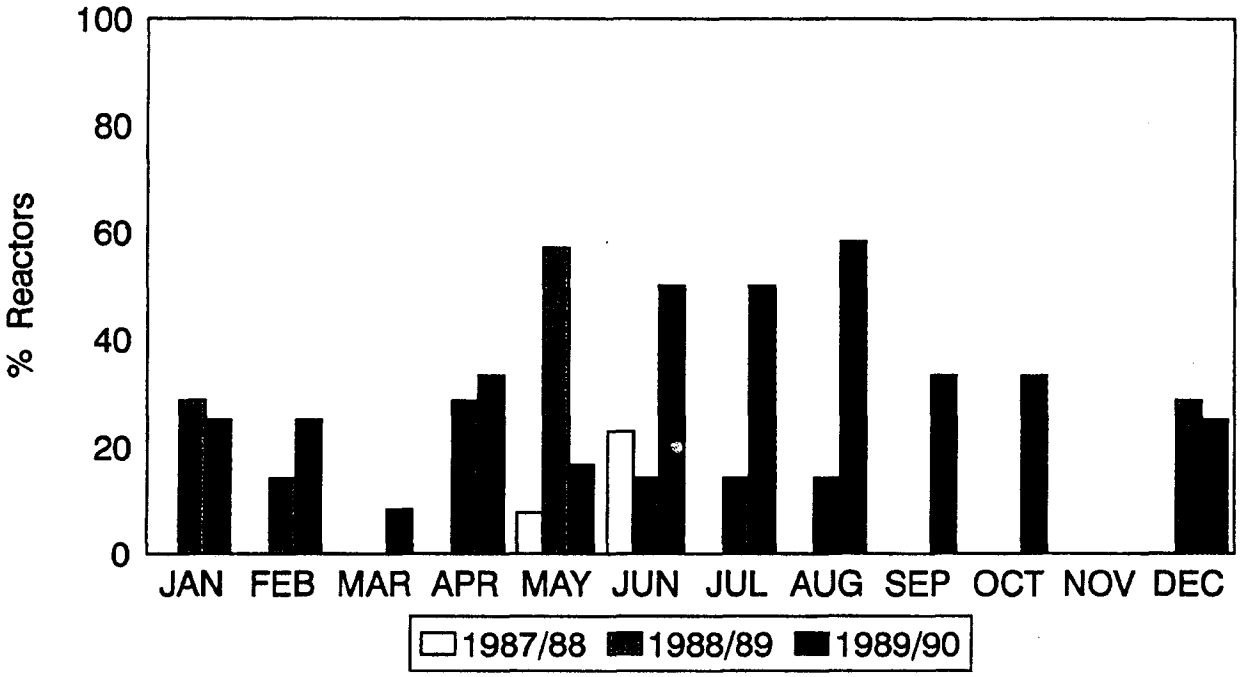

FIG.2. Percentage of reactors to BTV in sentinel cattle at Cisarua, a high altitude area, (June 1987 to 1990).

TABLE I. COMPARISON OF REACTIVITY OF SERA TO BTV IN AGID TEST AND ELISA

	ELISA	
AGID*	+	-
+	154	33
-	72	313

*BTV-20 used as antigen.

TABLE II. COMPARISON OF REACTIVITY OF SERA TO BTV IN AGID TEST AND COMPETITIVE ELISA

	ELISA	
AGID*	+	-
+	105	32
-	48	54

*BTV 1 used as antigen.

Comparison of the AGID and C-ELISA results indicated that C-ELISA detected maternal antibody longer than did the AGID test. Maternal antibodies were detected for 3 months in the AGID test and then declined to undetectable levels. However, using C-ELISA maternal antibodies were still detected up to 6 months of age before declining to less than the 40% inhibition level cut-off point. After the animals were naturally infected, seroconversions were detected earlier by C-ELISA and

before the AGID became reactive. Isolation was attempted to obtain BTV isolates. From 469 samples processed from Depok, 18 isolates (3.8%) were obtained by inoculating directly into BHK-21 cells. The isolation method was improved by inoculating into embryonated chicken eggs before passaging into *Aedes albopictus* and BHK-21 cells. The latter method yielded 34 isolates from 477 blood samples processed (7.1%). However, these two methods could not be directly compared because the samples used in two methods were not the same. Isolates were not obtained from the high altitude area.

Insects were collected for identification. Twenty one *Culicoides* spp. were found in the low altitude area including *C. barnetti, C. parahumeralis, C. palpifer, C. albibasis, C. gewertzi, C. actoni, C. flavipunctatus, C. fulvus, C. orientalis, C. wadai, C. brevitarsis, C. jacobsoni, C. peregrinus, C. sumatrae, C. insignipenis, C. guttifer, C. arakawae, C. oxystoma, C. shortii, C. geminus, C. huffi.* Four of these species, *C. albibasis, C. flavipunctatus, C. brevitarsis,* and *C. arakawae* were not found in the high altitude area during the 1 year of observation (1990-1991).

Isolation from insects was also attempted. Serotyping indicated that a pool of the Avaritia subgenus which comprised 10% *C. orientalis* and 90% *C. fulvus* yielded BTV serotype 21 [9]. From 52 isolates obtained from cattle blood and insects, some were processed for group identification. Initial results identified 21 isolates of the BTV group. Confirmation of isolates and serotyping were conducted by the OIE Reference Laboratories for bluetongue virus at Onderstepoort, South Africa and Pirbright, United Kingdom. At present, these laboratories have confirmed 6 BTV serotypes, 1, 7, 9, 12, 21 and 23 from West Java [6, 13].

4. DISCUSSION

The studies reported here were part of a wider study of the epidemiology of arboviruses in Indonesia which includes serological surveys, monitoring of sentinel animals, and vector studies [14].

Some sera did not react in the AGID test but were positive in C-ELISA (Tables I and II). Some of those sera contained maternal antibodies that were detected longer by C-ELISA, while others represented early responses to infections. Maternal antibodies were detected for up to 3 months in the AGID, and then declined to undetectable levels while still being detected in the C-ELISA for up to 6 months. This means the C-ELISA detected low titres of antibodies which the AGID did not. These results were consistent with those obtained earlier by Anderson [7] and Afshar *et al.* [10], who demonstrated that a C-ELISA using Mab 3-17-A13 detected low titres of BTV antibodies and maternal antibodies up to 6 months. Analysis of results obtained from individual animals indicated that seroconversions were detected earlier by the C-ELISA and before the AGID became reactive. Again, this may indicate better sensitivity of the C-ELISA.

The results also indicated that some sera were positive in the AGID but negative in the C-ELISA. This suggests that these calves were exposed to other related orbiviruses such as EHD, Palyam group viruses or Eubenangee [15]. However, it cannot be proved that reactions in the AGID test were due to such other viruses as reagents for their study were not available. However, Anderson [7] demonstrated that there is little or no inhibition of the BTV monoclonal antibody reactivity by EHD virus antibody, but that high levels of inhibition occurred with antibodies to all BTV serotypes tested. Other workers confirmed that monoclonal antibodies specific to BTV group did not react with antibodies to other orbiviruses such as EHDV in the C-ELISA [16].

In the 3 years of observation, seroconversions mostly occurred in February-March at the low altitude site. However, in the high altitude area seroconversions occurred mostly in May-June. Based

on historical monthly rainfall data [17], February-March at low altitude is usually the end of the wet season, while in the high altitude area the end of the wet season is usually in May-June. Hence there appears to be a trend for seroconversions to occur at the end of the wet season.

Based on the serological data, BTV infections were more prevalent at low altitude than in the high altitude area. Also BTV serotypes 1, 7, 9, 12, 21 and 23 [6, 12, 13] were isolated at Depok, but no isolates were obtained at the high altitude area. These differences may be related to the vector populations at each site. At Cisarua, fewer *Culicoides* spp. were trapped than at Depok. At the lower site the temperature is warmer (average 28°C compared with 20°C), and sanitation is poorer (huge dung piles near the animals may act as breeding sites for *Culicoides* spp). Hence a combination of broad climatic influences and local environmental factors may influence the abundance of vectors and hence the incidence of infections.

During one year of observation on sentinel goats and sheep, seroconversion did not occur. Previous serological surveys indicated that small ruminants had a lower prevalence of reactors than large ruminants [2]. Again, local environmental factors may have contributed to an apparent lack of BTV infections. Animals were not grazed but were housed in pens under roofs. Some *Culicoides* spp. do not enter such pens. Also cattle and buffaloes were not found in the adjacent area perhaps due to the hilly nature of the land and so were not present to act as amplifier hosts for virus or to support cattle during breeding *Culicoides* spp. Insect collections trapped only a very small number of *Culicoides* spp. It is believed that the feeding preferences of *Culicoides* spp. also result in lower exposure of small ruminants to infections.

The isolation of BTV virus serotypes 1, 7, 9, 12, 21 and 23 confirms that BTV is present in Indonesia, even though clinical bluetongue disease has not been reported in indigenous ruminants. BTV serotypes 1, 21 and 23 were also reported from another province, Irian Jaya, which is some 4,000 km from Bogor [12]. BTV serotype 21 was isolated from a pool of *C. fulvus* and *C. orientalis*. In Australia, *C. fulvus* is a proven BTV vector suggesting that some vectors may be the same in the two countries. However, further detailed studies of BTV vectors in Indonesia should be conducted. This work should be followed by studies on the pathogenesis of Indonesian BTV isolates and studies to determine whether Indonesian strains are different from those of other countries.

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REFERENCES

[1] ERASMUS, B.J., Bluetongue in sheep and goats, Aust. Vet. J., **51** (1975) 165-170.

[2] SENDOW, I., YOUNG, P., RONOHARDJO, P., Preliminary survey for antibodies to bluetongue -group virus in Indonesian ruminants, Vet. Rec., **119** (1986) 603.

[3] SENDOW, I., The agar gel immunodiffusion test for the detection of antibody to bluetongue group viruses in Indonesian ruminants, Penyakit Hewan **21** (1989) 59-64.

[4] SENDOW, I., DANIELS, P.W., CYBINSKI, D.H., YOUNG, P.L., RONOHARDJO, P., Antibodies against certain bluetongue and epizootic haemorrhagic disease viral serotypes in Indonesian ruminants, Vet. Microbiol., **28** (1991) 111-118.

[5] SENDOW, I., DANIELS, P.W., SOLEHA, E., SUKARSIH, Epidemiological studies of bluetongue viral infections in Indonesian livestock. In Bluetongue, African Horse Sickness and Related Orbiviruses, Eds. Walton, T.E., Osburn, B.I., CRC Press, Boca Raton. (1991) 147-154.

[6] SENDOW, I., DANIELS, P.W., SOLEHA, E., HUNT, N., RONOHARDJO, P., Isolation of bluetongue viral serotypes 7 and 9 from healthy sentinel cattle in West Java, Indonesia, Aust. Vet. J., **68** (1991) 405.

[7] ANDERSON, J., Use of monoclonal antibody in a blocking ELISA to detect group specific antibodies to bluetongue virus, J. Immunol. Meth., **74** (1984) 139-149.

[8] DELLA-PORTA, A.J., SELLERS, R.F., HERNIMAN, K.A.J., LITTLEJOHN, I.R., CYBINSKI, D.H., ST GEORGE, T.D., McPHEE, D.A., SNOWDON, W., CAMPBELL, J., CARGILL, C., CARBOULD, A., CHUNG, Y.S., SMITH, V.W., Serological studies of Australian and Papua New Guinean cattle and Australian sheep for the presence of antibodies against bluetongue group viruses. Vet. Microbiol., **8** (1983) 147-162.

[9] SENDOW, I., SUKARSIH, SOLEHA, E., ERASMUS, B.J., DANIELS, P.W., Isolation of bluetongue virus serotype 21 from *Culicoides* spp in Indonesia, Vet. Microbiol., in press (1993).

[10] AFSHAR, A., THOMAS, F.C., WRIGHT, P.F., SHAPIRO, J.L., ANDERSON, J., FULTON, R.W., Blocking dot ELISA using a monoclonal antibody for detection of antibodies to bluetongue virus in bovine and ovine sera, J. Virol. Meth., **18** (2987) 271-279.

[11] CYBINSKI, D.H., ZAKREZWENSKI., The isolation and preliminary characterization of a rhabdovirus in Australia related to bovine ephemeral fever virus, Vet. Microbiol., **86** (1983) 221-235.

[12] SENDOW, I., SOLEHA, E., DANIELS, P.W., SEBAYANG, D., ACHDIYATI, J., KARMA, K., ERASMUS, B.J., Isolation of bluetongue viral serotypes 1, 21 and 23 from healthy sentinel cattle in Irian Jaya, Indonesia, Aust. Vet. J., in press (1993).

[13] SENDOW, I., DANIELS, P.W., SOLEHA, E., ERASMUS, B., SUKARSIH, RONOHARDJO, P., Isolation of bluetongue virus serotypes new to Indonesia from sentinel cattle in West Java, Vet. Rec., in press (1993).

[14] DANIELS, P.W., SENDOW, I., SOLEHA, E., JENNINGS, J., SUKARSIH, A veterinary arbovirus monitoring program. In: Proceedings 6th Congress of the International Society for Veterinary Epidemiology and Economics (ISVEE), Ed. S.W. Martin, Guelph, Canada (1991) 207-209.

[15] DELLA-PORTA, A. J., PARSONSON, I.M., McPHEE, D.A., Problems in the interpretation of diagnostic tests due to cross-reactions between orbiviruses and broad serological responses in animals, In: Bluetongue and Related Orbiviruses, Eds. Barber T.L., Jochim M.M., Alan R. Liss, Inc., New York (1985) 445-453.

[16] LUNT, R.A., WHITE, J.R., BLACKSELL, S.D., Evaluation of a monoclonal antibody blocking ELISA for the detection of group-specific antibodies to bluetongue virus in experimental and field sera, J. Gen. Virol., **69** (1988) 2729-2740.

[17] Anon. Rainfall Observation in Indonesia. 1961-1970, Institute of Meteorology and Geophysics, Department of Communications, Jakarta (1975).