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Review

Australian-Indonesian collaboration in veterinary arbovirology – a review

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Abstract

Australian-Indonesian collaboration in veterinary development programs has led to significant advances in the study of arboviruses. This paper reviews the resulting knowledge of arboviral infections of livestock in Indonesia. The first recognized arboviral disease of animals in Indonesia was bovine ephemeral fever. Serology indicates that the virus is widespread, as are related rhabdoviruses. Local sheep appear resistant to bluetongue disease, but imported sheep have suffered mortalities. Bluetongue viral serotypes 1, 7, 9, 12, 21 and 23 have been isolated from sentinel cattle; 1, 21 and 23 at widely separate locations. Bluetongue serotype 21 has been isolated from *Culicoides* spp. Serological reactors to Akabane virus are widespread, as are reactors to the flavivirus group. Japanese encephalitis, isolated from sentinel pigs, is the flavivirus of most veterinary importance but the limit of its easterly distribution is unknown. Many of the arboviruses present in Indonesia are also present in Australia and elsewhere in Asia. Their patterns of mobility among countries in the region are largely undescribed, but there are opportunities for further regional collaboration.

Keywords: Arbovirus; Bovine ephemeral fever; Bluetongue disease; Indonesia; Australia

1. Introduction

Indonesia is an archipelago spanning the Equator for 5600 km and being 1600 km north to south. It comprises over 13,000 islands lying between mainland Asia and Australia, with

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a land area of 1.8 m sq km. The human population is in excess of 180 m, but the country is largely self sufficient in food. Rural areas are representative of both the wet and dry tropical production systems. Livestock play an important role in the economy, and there are in excess of 10 m cattle, 3 m buffaloes, 5.5 m sheep, 11 m goats and 7 m pigs (Soejasmiran, 1990).

In a tropical country where insect life is abundant and mammalian hosts have large populations, arboviral infections can be expected to be prevalent. This paper reviews knowledge of the main arboviral groups, particularly noting the contributions of collaborative research between Indonesia and Australia. As arboviral infections are highly mobile, infected insects being spread by wind movements (Sellers, 1980), a collaborative regional approach to the study of arboviruses is suggested as offering the potential to provide the fullest epidemiological understanding of arboviral infections in both countries.

2. Australian-Indonesian collaborative programs

Australian–Indonesian programs in arbovirology have mostly been within the context of broader development collaboration. A major Australian International Development Assistance Bureau (AIDAB) funded program managed by James Cook University assisted institutional development at the Research Institute for Veterinary Science (Balitvet) in Bogor (Fig. 1) from 1981 to 1990. Balitvet is the central veterinary research and reference

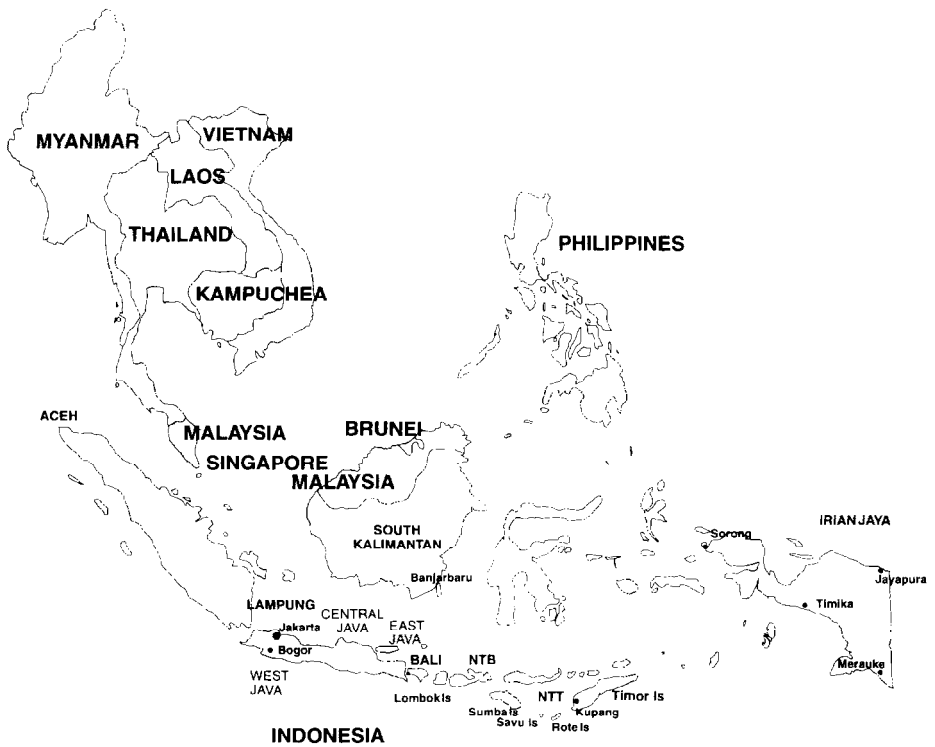

Fig. 1. Indonesia showing sampling locations.

laboratory within the Agency for Agricultural Research and Development of the Ministry of Agriculture. The laboratory developed expertise in virological techniques based on cell culture, established a serum bank (Young et al., 1985), developed an ELISA capability and produced monoclonal antibodies and applied PCR technology to the diagnosis of viral disease.

Serological studies of bluetongue (BLU) viruses were commenced (Sendow, 1988). Subsequently collaborative work was initiated with the United States Medical Research Unit (NAMRU) in Jakarta, transferring Australian sentinel herd technology (St. George, 1980) for the monitoring of sentinel cattle and pigs in West Java, South Kalimantan and Nusa Tenggara Timor (NTT) provinces (Fig. 1) (Sendow et al., 1988). Subsequently studies on the BEF group arboviruses were incorporated (Soleha, 1991). A fellowship from the International Atomic Energy Agency assisted the introduction of ELISA technology for BLU serology (Sendow et al., 1994a).

Priorities were reviewed and an overall strategy for veterinary arbovirology planned (Daniels, 1991). Surveys to determine distribution and the mammalian hosts were proposed. Establishment of sentinel groups in important livestock rearing areas would allow isolation of viruses and determination of seasonal patterns. Vector studies should include not only detection of virus in wild caught insects but also vector competence studies. Any association of viruses with disease should be established.

Epidemiological studies were expedited by a program with Australia under the Northern Australian Quarantine Strategy (NAQS). This commenced in 1989–1991 with weekly monitoring of sentinel animals accompanied by quarterly surveys in two districts in Irian Jaya province (Jayapura and Merauke), Kupang district in NTT, and sites in Bali and West Java provinces (Fig. 1) (Daniels, 1991; Daniels and Ginting, 1992; Daniels et al., 1994). A major study of potential vectors, the *Culicoides* spp., was included (Sukarsih et al., 1993). After the Balitvet Project finished in 1990, coordination of the NAQS program passed to the Indonesia International Animal Science Research and Development Foundation (INI ANSREDEF), created by Indonesian authorities to assist in post-project sustainability of externally funded programs at Balitvet (Anon., 1992).

In 1991/1992 the sampling intensity under NAQS was reduced, with sentinels monitored fortnightly for virus isolation at the Irian Jaya sites. Weekly collections continued at Kupang but INI ANSREDEF assumed responsibility for sampling in West Java. Sampling in Bali was discontinued in 1993/94, but during 1992/93 sentinel cattle were established for monthly serological monitoring and insect collection at additional sites in NTT, Waingapu in east Sumba Island, Rote Island and Attambua in Belu District of West Timor. Greater emphasis was placed on areas thought to be the source of movements of viruses and vectors.

Further epidemiological studies in the eastern islands of Indonesia were assisted by the Cattle Health and Productivity Survey (CHAPS) of the AIDAB funded Eastern Islands Veterinary Services Project (EIVSP) managed by NSW Agriculture (Christie et al., 1992). Quarterly samples for arbovirus serology were taken from groups of cattle at several sites in the provinces of Nusa Tenggara Barat (NTB) and East Timor as well as NTT (Sendow and Soleha, 1994; Soleha et al., 1994).

3. Bovine ephemeral fever group viruses

3.1. BEF Virus and other Rhabdoviruses

The rhabdoviruses have been broadly classified into two groups, the vesiculoviruses such as vesicular stomatitis virus and the lyssaviruses which comprise several serogroups, including the rabies, BEF and Tibrogargan serogroups (Calisher et al., 1989). Rabies is present in Indonesia, but there has been little information on the related lyssaviruses. There is only one vesiculovirus reported in south east Asia, Porton virus, isolated from *Mansonia uniformis* in Malaysia (see Calisher et al., 1989). Several of the BEF group have been isolated only in Australia, Adelaide River (AR) (Gard et al., 1984), Berrimah (BRM) (Gard et al., 1983) and Kimberley (KIM) (Liehne et al., 1981).

3.2. Historical aspects

The history of BEF in Indonesia has been reviewed by Daniels et al. (1993). The first report described a new disease in dairy cattle in Bandung, West Java, consistent with BEF (Merkens, 1919). Cases of "3 day sickness" were next observed as an epizootic between 1928 and 1931 on the east coast of Sumatra (Burggraff, 1932). A higher incidence occurred at the beginning of the wet season, when mosquitoes became abundant. Disease spread without direct contact, suggesting an insect vector.

In 1978 BEF reappeared, in East Java. The source of infection was attributed to *Bos indicus* cattle from Sumba island to the east where a similar disease had affected local *Bos indicus* cattle and *Bos indicus* cattle imported from Australia. In a serum neutralisation test on recovered cattle, 22 of 25 were reactors to BEF (Soeharsono et al., 1982). Mortalities were numerous over several years (Ronohardjo and Rastiko, 1982). A second serological study of 90 cattle from East Java and Bali found 79% reactive (Miura et al., 1982). Clinical cases were again observed in East Java in 1985 (Daniels et al., 1988a).

The first epidemic of BEF in northern Australia was in 1936, five years after the outbreak in Sumatra. The spread across northern Western Australia was quite rapid, properties along a 400 mile gradient being affected within a 2 week period. The outbreak was an incursion of an exotic disease (Seddon, 1938) and Sumatra was the nearest known point of infection. Entry to Australia via infected insects was suspected. Seddon (1938) discussed possible movements associated with coastal shipping and Mackerras et al. (1940) noted scientific evidence for the transport of mosquitoes and Culicoides spp on upper air movements. By 1940 Mackerras et al. (1940) considered the disease endemic.

3.3. Serological surveys

A serum microneutralization test for BEF was established (Soleha, 1991), and BEF serological studies were incorporated into the arbovirus program (Daniels et al., 1991). Although cross reactions are known to occur among BEF group viruses, even in serum neutralization (SN) tests (Gard et al., 1983), this approach has given a useful preliminary indication of BEF group viral activity.

Nationwide distribution

Reactors to BEF were detected from Aceh in the north of Sumatra Island in the west through to Irian Jaya in the east. Sero-prevalences of 13% to 38% were observed in samples of between 18 to 55 cattle from each of 12 of Indonesia's 29 provinces, indicating BEF viral infections were widespread (Daniels et al., 1993).

In 1991 there was a major outbreak of suspected BEF in South Kalimantan province. Disease was reported from 27 village areas in 5 districts of the province. Seroprevalences in a survey in January 1992 varied from 26% to 64%, being highest in the coastal district (64%) and varying from 26% to 36% further inland (Soleha et al., 1992a).

Species distribution in West Java

Antibodies to the BEF group rhabdoviruses known in Australia were detected in several species of livestock in West Java. Reactors to BEF were found in cattle (15%) and buffaloes (17%); to BRM in cattle (10%), buffaloes (29%), sheep (13%), and goats (3%); to KIM in cattle (20%), buffaloes (29%), goats (2%), and horses (8%); and to AR in buffaloes (2%) and goats (23%). The were no reactors among 36 ducks or 58 chickens sampled (Soleha et al., 1993b). These four viruses also infect cattle and buffaloes in Australia (Cybinski and Gard, 1986).

BRM and KIM differed from BEF in that antibodies were detected in small ruminants, sheep and goats (BRM) and goats (KIM). Antibodies to KIM were also found in horses, in contrast to Australia (Cybinski and Zakrzewski, 1983). Goats had a higher sero-prevalence for AR than the other viruses. In Australia AR antibodies were found in pigs but not in horses or goats (Gard et al., 1984). In Indonesia, the mammalian host range of the BEF group may be wider than previously indicated, although large ruminants were again implicated as the main hosts.

Surveys in Eastern Indonesia

Under the NAQS program there have been more intensive serological surveys of adult cattle in Irian Jaya and in NTT. The results confirm that BEF, or closely related viruses, are widely distributed in eastern Indonesia (Table 1) (Soleha et al., 1993a). In Irian Jaya, infections were more prevalent on the north coast than on the south coast. Seroprevalences in West Timor adjacent to northern Australia were among the highest observed in the study, up to 60% and 77% in 1993 (Soleha, unpublished).

Merauke (Irian Jaya) and Kupang (West Timor) have similar climates but Merauke is more isolated, separated from Jayapura by a wide mountainous area, where BEF antibodies were not detected in the few cattle present (Table 1). The cattle population of West Timor, where Kupang is located, is approximately 0.5 million compared with 10,000 in Merauke. However the cattle population on the south coast of Irian Jaya is increasing, with imports from NTT and NTB. Patterns of arboviral activity may change as populations of mammalian host increase.

The CHAPS serological survey contributed further information on BEF infections in Lombok and Sumbawa in NTB province, Sumba, Flores and West Timor in NTT province and from East Timor province (Christie et al., 1992). Seroprevalences were high, being 67% of 348 animals sampled from NTB, 60% of 420 from NTT and 60% of 124 from East Timor (Soleha et al., 1994), comparable with results in the NAQS program in 1992/93

Table 1
Prevalence of reactors to bovine ephemeral fever virus in cattle in eastern Indonesia – a comparison over several years

Province/District	1989 % Reactors (No. tested)	1990 % Reactors (No. tested)	1993 % Reactors (No. tested)
Irian Jaya			
Jayapura	24% (122)	24% (156)	33% (54)
Jayawijaya	0% (9)	–	–
Merauke	9% (86)	7% (128)	1% (93)
Fak Fak	2% (28)	–	3% (28)
Sorong	–	25% (28)	–
Biak Numfur	–	44% (25)	9% (12)
Nusa Tenggara Timur			
Belu	–	–	60% (40)
Kupang	17% (105)	42% (113)	77% (30)
Kupang (Savu Is)	–	–	13% (30)
Kupang (Rote Is)	–	–	26% (34)

(Soleha, unpublished) and in excess of those found previously among a smaller group of animals in the first nationwide survey (Daniels et al., 1993).

3.4. Seroconversions in sentinel cattle

West Java

BEF group antibodies have been detected each year since 1987, and sero-conversions have been observed in most months of the year (Soleha et al., 1993b).

Eastern Indonesia

Patterns of BEF group activity in groups of 10 *Bos javanicus* sentinel cattle in Bali, Kupang and Jayapura, approximately 1000 km apart, were compared (Daniels et al., 1993).

In Bali, with a sero-prevalence of 13% in the nationwide survey, calves seroconverted to BEF in 1989/1990. Not all animals seroconverted (Soleha et al., 1993a) and since calves seroconverted to different BEF group viruses at different times, it seems several different viruses were circulating.

Patterns in Kupang and Jayapura were similar to those in Bali. In Bali and Kupang seroconversions to BEF and BRM occurred first in December, and then in March, April and May. In Jayapura further to the east, seroconversions to these viruses were again first in December, and then in April and May reaching a peak in June and July. Although data are as yet limited there appears little indication of an easterly movement of infections. Since the wet season eases later in Jayapura (Anon., 1973), this may affect local conditions and delay some vector activity.

The CHAPS data from quarterly samplings again showed that seroconversions occurred at slightly different times from island to island, and hence the point prevalence for BEF reactors was dependent on the time of sampling (Soleha et al., 1994). In NTT seroconversions occurred during different months in successive years, indicating that the epidemiology

of BEF in the region can not be fully understood from a short study. Although the timing of seroconversions was somewhat different in NTB from NTT, there was again no evidence of an eastward spread of infections with the north-west monsoons.

3.5. Virus isolation

Seven isolates from sentinel cattle blood samples at Depok in West Java were related to the BEF group on the basis of reactions with antibodies to BEF strain BB7721 by indirect immunofluorescence (IIF). One of these isolates, from March 1990, reacted with antibodies to one of the four BEF group viruses under study, BRM, in a micro neutralisation test (Soleha, unpublished). Further two-way neutralisation tests are needed to confirm this identification. Since 6 of 7 isolates were not neutralised by antisera to the four prototype viruses other viruses related to BEF may have been isolated.

3.6. Epidemiological considerations

Since BEF-like disease occurs and serological reactors have been detected nationwide with seroconversions in sentinel cattle, then BEF virus may be considered endemic in Indonesia, subject to final confirmation by virus isolation.

Present studies have utilised only Australian members of the group. Other viruses are included in the BEF group, Malakal virus from the Sudan, and Puchong virus from Malaysia (Calisher et al., 1989). Because of geographical proximity to Malaysia, Puchong virus should be sought in Indonesia. Other undescribed BEF group viruses may also exist.

Although there has been intensive monitoring of yearlings as sentinel cattle over a number of years, disease has not been observed. However BEF-like disease still has been reported in the provinces under study (Daniels et al., 1994) and a large outbreak of suspected BEF was reported in South Kalimantan (Soleha et al., 1992). Although the first reported outbreak of disease (Merkens, 1919) was from a cooler area where exposure may be less frequent and hence a higher proportion of the host population naive (Soleha et al., 1993a), more recent outbreaks from East Java and South Kalimantan (Soeharsono et al., 1982; Soleha et al., 1992) were from hot tropical areas. The sero-prevalence found in surveys, mostly around 20%, and the patterns of only a few animals in sentinel groups seroconverting, suggest that in some areas the virus was not spread among susceptible animals with great efficiency.

4. The orbiviruses

A useful guide to the orbiviruses that may be present in the south east Asian region is the isolations in northern Australia, as reviewed by Daniels and Melville (1994). Among the more important are 8 bluetongue (BLU) serotypes, BLU 1, 3, 9, 15, 16, 20, 21, 23 and six epizootic haemorrhagic disease (EHD) serotypes, EHD 1, 2, 5, 6, 7, 8. In addition there are 4 Palyam group viruses identified and 5 other orbiviruses from the Corriparta, Wallal, Warrego and Eubenangee serogroups as well as the ungrouped Wongorr virus.

In Australia all of the BLU viruses were isolated first in the north, and 6 of the 8 have been isolated only from the far north of the Northern Territory. Furthermore, serological examination of stored sera indicates that for 5 of the 8 serotypes the viruses were detected within 2 years of their distribution in the north. There appears to be a pattern of occasional or recent introduction of these viruses from a neighbouring source (Gard and Melville, 1989). Identification of BLU viruses in Indonesia, to the immediate north and northwest of the main BLU infected areas in Australia, has therefore been of interest.

4.1. Historical aspects

The first report of suspected BLU in Indonesia was in imported Suffolk sheep from southern Australia (Sudana and Malole, 1982). A virus was isolated at the Disease Investigation Centre in Denpasar, but it subsequently failed to regrow in cell culture (Young, personal communication). BLU disease has not been reported in local sheep (Adjid and Daniels, 1993).

Other BLU and EHD viruses were isolated by NAMRU, as a result of arboviral studies in mosquitoes, but these were not serotyped (Brown et al., 1992). The EHD isolates were from *Anopheles vagus* collected on Bali in 1981 and the BLU isolate was from *Anopheles vagus* collected in Central Java in November 1981. A unique but ungrouped orbivirus was also isolated from *Anopheles subpictus* mosquitoes collected in Flores in NTT province in January 1981. The name Golok was proposed (Brown et al., 1992).

Early serological investigations of BLU were limited by small numbers of sera. Agar gel immunodiffusion (AGID) test reactors were found in East Java in cattle and buffaloes (Sudana and Malole, 1982) and Herniman et al. (1980) also reported bovine reactors in Indonesia.

In SN tests of cattle sera collected in 1979 in Java and Bali 54% of 82 reacted to BLU serotype 1, 4% of 77 reacted to BLU serotype 12 and 65% of 72 reacted to BLU serotype 20. No antibodies were detected against EHD 1 or Eubenangee, whereas seroprevalences were high to EHD 2 (72%), Mitchell River (100%) and Warrego (40%) (Miura et al., 1982). Neutralizing antibodies to Ibaraki virus had been reported previously in Bali (Inaba, 1975), with Ibaraki virus subsequently being suggested to be synonymous with EHD 2 (Campbell and St. George, 1986). EHD 2 and EHD 5 were the first EHD viruses to be isolated in Australia, in the Northern Territory in 1980 and 1979 respectively (St. George et al., 1983), and hence EHD 2 appears to have been widely distributed throughout the region.

4.2. Isolation of viruses

The first isolations of BLU viruses were from groups of sentinel cattle in West Java and NTT (Sendow et al., 1988). A virus reacting with antisera to BLU in a gel test was isolated from NTT in March 1988, as yet not further characterised. Retesting of these isolates and others with a broader range of antisera (Sendow et al., 1989) gave preliminary identification of two Palyam serogroup isolates from Kupang with a cross reaction with the EHD serogroup in one case. Two isolates from Depok in West Java were subsequently confirmed as BLU serotypes 7 and 9 by Pirbright (Sendow et al., 1991a). Further isolates from Depok reacted

with either the Palyam or EHD serogrouping fluids, while two others reacted with both Eubenangee and Akabane antisera (Sendow et al., 1989).

A functioning sentinel system both at Depok in West Java, near Balitvet, and at Kupang in NTT, remote from the laboratory, was in place when the NAQS program began seeking avenues for collaboration in Indonesia. Sites at Jayapura and Merauke in Irian Jaya and Denpasar in Bali were added to the program. Daniels et al. (1994) gave a summary of the isolates in the period 1989 to June 1993. From 2143 blood samples processed at the time from Kupang and the Irian Jaya sites, 65 isolates as evidenced by CPE in BHK 21 cell culture (after primary I/V inoculation of embryonated eggs and passage in C6/36 cells) were recorded. Forty were from Jayapura, 19 from Kupang and 6 from Merauke. Bali yielded 14 isolates and 33 isolates had been obtained from 737 blood samples processed from Depok collections (Sendow, unpublished).

Only a small proportion of these have yet been characterised. Preliminary identifications of BLU group isolates was given by Sendow et al. (1993c). There had been 18 suspect BLU viruses recovered from Depok samples, 2 from Kupang, and 11 from Jayapura, based on reactions with the BLU group monoclonal 20E9/B7/G2 from AAHL (Lunt et al., 1988) in an immuno-dot blotting system. Twelve isolates had also been obtained from 390 insect pools collected at Depok, and 4 of these typed as BLU group viruses (Sendow et al., 1993c).

In 1990 BLU viral isolates from Depok sentinel cattle were confirmed by Onderstepoort. Five BLU serotypes (1,9,12,21,23) were isolated from the one sentinel group over a 6 month period (Sendow et al., 1993a).

At Jayapura, 4000 km east of Depok in West Java, similar serotypes were isolated during the same period (Sendow et al., 1993b). The temporal relationship of these isolations was such that no movement of viruses from west to east with the prevailing monsoons could be suggested, and it appears that the BLU viruses are endemic in both areas. Since BLU viruses have been confirmed from Depok in 1988 and 1990 (Sendow et al., 1991a; 1993a) and identified at other times (Sendow, unpublished) Depok seems a useful area for the study of arboviruses, perhaps analogous to the Coastal Plains site which has been confirmed as a site of above average arbovirus activity in Northern Australia (Daniels and Melville, 1994).

Presumed arboviruses have been isolated from pools of the Trithecoides subgenus of *Culicoides* (5), pools of the Avaritia subgenus (2), *C. fulvus* (1), *C. maculatus* (1), *C. orientalis* (1) and *C. peregrinus* (1) as well as a pool of Aedes spp mosquitoes (1) (Sukarsih, unpublished). The isolates from one pool of Avaritia, *C. fulvus* and the *Aedes* spp reacted in BLU grouping tests (Sendow, unpublished). The isolates from the Avaritia pool and the Aedes pool were subsequently each identified as BLU serotype 21 (Sendow et al., 1993d; Sendow et al., 1994b). This Avaritia pool contained 227 *C. fulvus* and 20 *C. orientalis* (Sendow et al., 1993d).

4.3. Serological surveys

Serological studies using the agar gel immunodiffusion (AGID) test detected reactors in cattle, buffaloes, goats and sheep in specimens collected from the islands of Sumatra, Java, Kalimantan, Sulawesi and Timor. Cattle had an average seroprevalence of 62%, buffaloes 75%, goats 37% and sheep 19% (Sendow et al., 1986).

AGID reactors were serotyped against BLU serotypes 1, 12, 17, 20 and 21 and EHD serotype 5 (Sendow et al., 1991b). The number of sera tested against each antigen varied slightly but was approximately 700 to 950 sera. Reactors to BLU serotype 20 were most prevalent (23%), with 21% reactors to EHD 5. There was a 6.5% seroprevalence to BLU 21, 2% to BLU 1, 4.5% to BLU 12 and 1.5% to BLU 17.

Sera with antibodies to BLU 17 reacted to BLU 20 with a higher titre, and the possibility of cross reactions was discussed. Cross reactions were also considered in the case of the 4.5% BLU 12 reactors, but the subsequent isolation of BLU serotype 12 from West Java (Sendow et al., 1993a) suggests that the reactions reflected actual infections. BLU 12 reactors were found only in Central Java and West Timor.

BLU 1 antibodies were detected only on Java in this study, but BLU 1 has been isolated subsequently in Irian Jaya. BLU 20, BLU 21 and EHD 5 antibodies were reactors on all of the islands in this study.

The national distribution of BLU serotypes 7 and 9 was tested with sera from adult cattle. Overall seroprevalences were low, 0.9% for BLU 7 and 4.6% for BLU 9. Antibodies to BLU 7 were detected on Sumatra, Java and Timor. Antibodies to BLU 9 were detected only in samples from eastern Indonesia, even though the isolation was from sentinel cattle in West Java (Sendow et al., 1992).

The AGID test was replaced by the C-ELISA test for the BLU group using antigens and monoclonal antibody supplied under an FAO/IAEA program (Sendow et al., 1994a). The C-ELISA was confirmed to be more sensitive that the gel test, as described previously (Anderson, 1984). Subsequently BLU C-ELISA tests were bought as kits (JCU Tropbio, Townsville) utilising reagents produced at AAHL (Lunt et al., 1988).

Surveys in villages in Irian Jaya were conducted. Seroprevalences for BLU group viruses were lower on the south coast than on the north, being 30% and 44% in two villages in the Merauke district and 27% in a village near Timika in Fak Fak district compared with 84% and 100% in villages in Jayapura district (Sendow, unpublished).

Cattle monitored for CHAPS in NTB, NTT and East Timor (Christie et al., 1992) were also tested (Sendow and Soleha, 1994). Prevalences of reactors were high, particularly in adult animals. Maternal antibodies were detected in calves, and seroconversions were detected as these antibodies waned.

4.4. Seroconversions in sentinel cattle

The results of monitoring from 1987 to 1990 were reviewed by Sendow et al. (1992). Historical rainfall records indicated the main peaks of seroconversion at each of 2 sites in Java were occurring in the mid to late wet season and the early dry season. Patterns were not so well developed in Timor. Seroconversions were not as prevalent and varied from year to year. In Jayapura, where there is usually over 150 mm rain every month, seroconversions were also detected in most months of the year.

4.5. Epidemiological aspects

Two apparently different patterns of BLU infections in Indonesia have been identified. There are widely distributed infections, as evidenced by the isolation of BLU serotypes 1,

21 and 23 from both West Java and Irian Jaya in early 1990 (Sendow et al., 1993a,b) and by the widely distributed reactors to BLU 20 and BLU 21 in SN tests (Sendow et al., 1991b). Conversely, on the basis of serological results infections with some serotypes appear to be restricted such as those with serotypes 7 and 9 (Sendow et al., 1992), and serotypes 1 and 12 (Sendow et al., 1991b).

Since the local breeds of sheep in Indonesia have not shown disease the BLU viruses have not yet had recognised economic impact. The situation in Malaysia is different, for there mortalities in imported Australian sheep have caused losses in an emerging farming system (Chiang, 1989; Hassan, 1992). There is concern that if Indonesia should also attempt fattening of imported Australian sheep then BLU disease may become important. Hence pathogenicity testing of Indonesian isolates in Australian Merino sheep has commenced (Daniels et al., 1994). Preliminary results indicate pathogenicity similar to that of Australian strains (Sendow and Pearce, unpublished).

5. Akabane virus and the bunyaviridae

5.1. Historical aspects

Of the Bunyaviruses, Ingwavuma (ING) has been isolated in Riau Province, Sumatra, from three species of mosquito (*Culex vishnui, Culex gelidus and Mansonia uniformis*) collected in 1982. Antibodies were found in 35 of 79 (44%) buffalo sera sampled (Converse et al., 1985). The clinical significance is undetermined. ING has been placed in the Simbu serogroup of the genus *Bunyavirus* (Beaty and Calisher, 1991).

The only other known isolate of the Bunyaviridae in Indonesia is Kao Shuan virus, isolated from *Agas robertsi* ticks (Karabatsos, 1985). Kao Shaun virus is in the Dera Ghazi Khan serogroup of the genus *Nairovirus* (Beaty and Calisher, 1991), and has also been isolated from *Agas robertsi* in northern Australia (Karabatsos, 1985).

Miura et al. (1982) conducted SN tests of Indonesian sera against a number of Bunyaviruses from several geographic origins (Karabatsos, 1985), with few clear patterns emerging. There were high seroprevalences to viruses widely distributed in Asia-Aino (99%), Akabane (AKA) (80%) and Batai (53%). There was also a high reactor rate to Ilesha virus (93%) normally distributed in equatorial Africa, and relatively low seroprevalences to viruses from central and South America-Kairi (30%) and Wyeomyia (3%). There were widely disparate prevalences to Australian members of the family -Tinaroo (68%), Peaton (58%) Douglas (12%), Wongal (6%) Trubanaman (16%), Kowanyama (57%) and Belmont (36%). Batai, Ilesha, Kairi and Wyeomyia are in the Bunyamwera serogroup of the genus *Bunyavirus*, AKA, Aino, Douglas, Peaton and Tinaroo are in the Simbu serogroup, Wongal is also a *Bunyavirus* (Koongol serogroup), while Trubanaman, Kowanyama and Belmont are less well defined (Beaty and Calisher, 1991). The higher reactor rates were to viruses known to be distributed elsewhere in Asia.

There is other serological evidence that Batai infects cattle and buffaloes (Olson et al., 1983). Reactors to AKA have been identified by SN tests in Bali and East Java (Sudana and Miura, 1982) and in Southern Sumatra and Lampung by HI tests (Marfiatiningsih, 1983).

5.2. Serological studies

A serological capacity for AKA has only recently been developed at Balitvet (Sendow, personal communication).

5.3. Epidemiological aspects

The Simbu group are viruses for which *Culicoides* spp., and particularly *C. brevitarsis*, can function as vectors. Since this insect is abundant in northern Australia and Indonesia it is possible that the two countries could share common populations of these viruses. The lower seroprevalence to Douglas (Miura et al., 1982) than the other Simbu group viruses may be a little surprising, especially as Douglas was the only Simbu group virus detected serologically in Papua New Guinea (Cybinski, 1984). These viruses may circulate at different times or in different years.

6. The alpha and flaviviruses

The alpha and flaviviruses in the South East Asian/Australian region are primarily associated with diseases of man, although domestic animals are infected and Japanese encephalitis (JE) is recognised as a zoonosis. For studies on the alpha and flaviviruses the Department of Health in Jakarta has for many years collaborated with NAMRU. A review was conducted for NAQS by Daniels and Ginting (1992).

6.1. Historical aspects

Epidemics of dengue fever-like disease have been reported in Indonesia since at least 1780, with clinical cases of suspected dengue (DEN) and JE disease being better documented since 1945. The first serological investigation of arboviruses in Indonesia, by the Bio Farma Institute at Bandung, was to DEN viruses. No isolations of arboviruses were known at that time (Van Peenen, 1971).

6.2. Isolation of viruses

Japanese encephalitis

The first isolation of an arbovirus in Indonesia was of JE from specimens of *Culex tritaeniorhynchus* mosquitoes collected by NAMRU near piggeries outside Jakarta in 1972 (Van Peenen et al., 1974a). Sentinel pigs were monitored from June 1972 to January 1974, and more mosquitoes collected. More isolates of JE were made from 11 *Culex tritaeniorhynchus* pools, and from one pool of *Culex gelidus* (Van Peenen et al., 1975a).

Isolations were primarily during the middle to late wet season (January to April) and not during periods of peak abundance of the mosquitoes (May to July) but JE was isolated from sentinel pigs in both March and June.

JE viruses have more recently been isolated from sentinel pigs near Bogor, West Java, under the collaborative program between Balitvet and NAMRU (Jennings, Sendow, Tan and Daniels, unpublished).

JE has also been isolated from the island of Lombok. In March, 1979 JE was isolated from three separate pools of *Anopheles vagus*, *Anopheles annularis* and *Culex tritaeniorhynchus* associated with buffaloes (Olson et al., 1985).

Dengue viruses

The flaviviruses of most medical significance in Indonesia are DEN viruses types 1 to 4. Type 1, 2 and 3 were first isolated in Jakarta in the early 1970s, while type 4 was first isolated in Medan in North Sumatra province from human blood specimens collected in 1973 (Van Peenen et al., 1974c, 1975b). They are now frequently isolated from cases of human disease.

Since Indonesia is an endemic area, human patients are frequently young children. However where foreigners from non endemic countries live in Indonesia for extended periods, severe clinical cases in adults are not uncommon. An outbreak of dengue fever in the Caucasian population of Bogor was monitored by the James Cook Project in collaboration with NAMRU. Between July 1987 and June 1988 15 of an estimated population of 100 adult expatriates had clinical disease consistent with DEN virus infection. DEN viruses type 1 and 2 were isolated (Daniels et al., 1988b).

Chikungunya virus

Chikungunya (CHIK) has emerged as the main alphavirus present in Indonesia. It is in the same group of viruses as Ross River (RR) virus in Australia. There have been isolations of CHIK from Central Java, from man and mosquitoes, and from Jakarta and South Kalimantan from human patients (Bartz, Jennings, personal communications).

6.3. Serological studies

One of the earliest serological studies of arboviruses in Indonesia was conducted in 1963 in the Jakarta area (Green et al., 1973). Human sera were collected from 4 different districts, and 24% of 479 sera were reactive to JE. Antibodies to JE were highest in the pig-raising district, 61% of the adult human population sampled being reactive.

Alphaviral antibodies were also detected, with 28% of sera being reactive to CHIK. These were predominantly high titre reactions. Antibodies to another group member, Sindbis, were of low titre and thought to be heterotypic responses.

Serological surveys of domestic animals were conducted to define mammalian hosts and potential amplifying hosts for some zooneses, including JE. In an abattoir survey of 399 pigs in the Jakarta area (Koesharjono et al., 1973) most had antibodies to JE in a haemagglutination inhibition (HI) test, with 70% being of high titre (1:80 or above). Pigs from Central Java also had a high seroprevalence. In a similar survey of cattle at a Jakarta abattoir, sera were collected from 399, 333 being from East Java. Thirty eight percent reacted in the HI test for JE, but only a small percentage at a titre of 1:80. It was concluded that cattle were not as an important mammalian host as pigs for JE (Van Peenen et al., 1974b), although the seroprevalence of JE in pigs in East Java was not reported.

Subsequently it was noted that differentiation of antibody responses to DEN and JE was not possible, particularly in HI tests but even with plaque reduction neutralisation tests (Van Peenen et al., 1974d). Hence previous data could have resulted from infections with either JE or DEN viruses. Since JE had been isolated from pigs and mosquitoes caught near pigs, antibodies in pigs probably did indicate JE infections.

The results from 3 studies in domestic animals –Miura et al. (1982) in East Java/Bali, Olson et al. (1983) in Lombok, and Gandahusada et al. (1984) in Lampung –were consistent. Flaviviral reactors in cattle had 35% to 47% prevalence, while chickens had 0% to 1% reactors. There was 0% to 1% reactors to alphaviruses in cattle at all 3 sites. Interestingly, in Australia, chickens are considered a useful sentinel for the flavivirus MVE, though are not as good a sentinel for the alphavirus RR (Kay et al., 1986, Broom et al., 1989).

6.4. Epidemiological considerations

The significance of alpha and flaviviral infections for Indonesian livestock has yet to be determined. It should be expected that pigs may suffer disease from JE from time to time, and that imported pigs may be more susceptible while naive.

JE is also a major pathogen causing mortalities in man in Indonesia, but as its distribution has not been determined its proximity to Australia is unknown. Isolations from Lombok (Olson et al., 1985) indicate spread east of the Wallace line (Wallace, 1869). Wallace himself noted that although much of the fauna from Lombok east to Timor was distinguishable from Javanese and Australian fauna, there were more mobile species of birds and insects common throughout the islands. Hence, as noted by St. George (1993), it would seem incorrect to hypothesise a protective natural barrier to the eastward, or westward, spread of insects and their arboviruses. These may be expected to appear wherever there are suitable mammalian hosts and the other ecological factors necessary for vector life cycles. The arrivals of certain *Culicoides* spp and the BLU and BEF viruses in northern Australia at some point after the introduction of large ruminants (St. George, 1986; St. George, 1992) are examples.

7. Vector studies (Culicoides spp.)

Collaborative studies with Australia have been of *Culicoides* spp., the presumed major vectors of BLU and other arboviruses of livestock in Indonesia. Historical entomological studies of the *Culicoides* in South East Asia have been extensively reviewed elsewhere (Wirth and Hubert, 1989).

The *Culicoides* spp. are a diverse group distributed globally, and have been largely subdivided into subgenera (Wirth and Hubert, 1989). Known BLU vectors are predominantly from the subgenus Avaritia: *C. actoni*, *C. brevitarsis*, *C. fulvus* and *C. wadai* in Australia and *C. imicola* in Africa. In Central America the main vector is *C. insignis* from the subgenus Hoffmania, while in North America the main vector is *C. variipennis* of the subgenus *Monoculicoides* (Standfast et al., 1985; Wirth and Dyce, 1985; Greiner et al., 1992).

Although several criteria to be met in identifying insects as arboviral vectors were noted in program planning (Daniels et al., 1991), under the NAQS project the first step was to describe the species present, and particularly to ascertain whether those known as vectors elsewhere in the region are present (Sukarsih et al., 1993).

7.1. Distribution of Culicoides spp

From light traps set at sentinel sites 49 species of Culicoides have been collected (Sukarsih et al., 1993). *C. brevitarsis*, *C. fulvus*, *C. flavipunctatus*, *C. orientalis*, *C. peregrinus* and *C. oxystoma* were found in all districts sampled.

West Java

Culicoides collected at Depok included 24 species. *C. sumatrae* was the dominant species, followed in decreasing order of abundance by *C. oxystoma*, *C. fulvus*, *C. peregrinus*, and *C. actoni*. At Cisarua, a higher altitude site in the mountains inland from Bogor, *C. parahumeralis* was the dominant species, followed by *C. fulvus*.

Bali

At Denpasar 19 species were collected. *C. peregrinus* was by far the most abundant species, followed by much fewer numbers of *C. oxystoma*.

West Timor

In Kupang 24 species were collected. *C. oxystoma* predominated, with *C. brevitarsis*, *C. histrio*, *C. peregrinus* and *C. geminus* also being well represented in collections.

Irian Jaya

At Jayapura 32 species of *Culicoides* were collected at the sentinel cattle site. *C. peregrinus* was the most abundant with *C. brevitarsis* second. In Merauke, *C. peregrinus* was the most abundant species among a total of 22 species in the collections, followed again by *C. brevitarsis*.

7.2. Seasonal fluctuations

The monthly totals of *Culicoides* spp. collected in light traps were compared to give an indication of seasonal abundance, although many factors can affect the size of catches leading to considerable day to day variation. At Depok there was a small increase in numbers in September, with a more substantial peak in January/February and a major peak in April. The increase in numbers in September was associated with an increase in *C. oxystoma*. The numbers of *C. peregrinus* increased slightly in January, and in April a proliferation of *Avaritia* spp occurred. Catches peaked at Cisarua a month later than at Depok.

There was year to year variation in the numbers of *Culicoides* trapped at Denpasar and in Eastern Indonesia as well as variation with site.

7.3. Epidemiological considerations

C. brevitarsis and *C. fulvus*, proven to be vectors in Australia, were present at all sentinel sites. Two *Avaritia* that feed on livestock but not present in Australia are *C. orientalis* and *C. nudipalpis* (Standfast et al., 1992). *C. orientalis* was widely distributed while *C. nudipalpis* was collected in eastern Indonesia. If these species are vectors of any BLU serotype their spread within the region may be associated with new risks.

C. peregrinus, also abundant at study sites, is in the subgenus *Hoffmania* that also includes the Central American vector *C. insignis*. It has been found to support replication of BLU after feeding on viraemic sheep (Standfast et al., 1985).

In general, seroconversions to BLU in West Java have occurred at the end of the wet season, at about the time that the *Avaritia* populations peaked (Sendow et al., 1989; 1994a). It remains to correlate these observations of insects with actual seroconversions and isolation of viruses from sentinel cattle.

Culicoides spp known as viral vectors in Australia have been identified in all areas studied and in Eastern Indonesia *C. brevitarsis*, a major Australian arbovirus vector, is the second most frequently trapped species. The finding of such species in the islands adjacent to northern Australia is a prerequisite for the theory that wind-borne movements of vectors between countries could occur.

8. A regional perspective

Australian veterinarians have been aware of the probability of arboviruses entering northern Australia from elsewhere. The routes of such suspected vector and arbovirus movements have been frequently hypothesised (Dyce, 1982; St George 1986; 1992), but remain unproven. Molecular studies of viral isolates are identifying differences among isolates of the same virus or serotype from different geographical regions, and technological developments are leading to the possibility that the origin of individual isolates may be identifiable (Gorman et al., 1981; Gould, 1987). For the BLU viruses, differences have been shown between Australian, South African and North American isolates, which have genetic relationships based on country of origin independently of serotype (Gould and Pritchard, 1991). Much could be learned of the relationships of BLU viruses in the Australian-South East Asian region by studying an adequate number of isolates from each country.

In Indonesia there is also an awareness of other regional aspects in animal health issues. In arbovirology it is noted that the *Culicoides* spp. feeding on livestock have moved in association with livestock across the normal boundary between Oriental (Indo-Malayan) and Australian (New Guinean) fauna, Weber's line (Dyce, 1982), into Irian Jaya (Sukarsih et al., 1993). BLU viruses have also now spread to this eastern region (Sendow et al., 1993d) and there is serological evidence that other viruses such as the BEF group (Daniels et al., 1993) are also present. Future molecular studies may show whether the viruses in Irian Jaya are homologous with isolates from Java. If so they may be recent immigrants from western Indonesia, as are many of the people and their livestock.

A broader regional perspective encompassing other countries is appropriate. As well as the BLU isolates from Australia and Indonesia, Malaysia has also reported isolation of BLU

viruses (Sharifah et al., 1994). A comparison (Table 2) shows many of the serotypes are common to all three countries, while some are presently unique to a particular country. However the full pattern of their distribution is probably not yet determined. There is scope for multilateral collaboration to learn even more of this presumed shared population of viruses. Beyond Malaysia, the nearest country reporting BLU viruses is India (Mehrotra, 1992; Uppal, 1992).

Among the other orbiviruses, EHD 2 has been isolated in both Japan and Australia (Campbell and St. George, 1986), with serologic evidence of infection in Indonesia (Inaba, 1975). A molecular comparison of Japanese and Australian isolates of EHD 2 showed them to be similar in sequence, suggesting both belonged to an Australasian grouping, or topotype (Gould and Pritchard, 1991).

BEF occurs in Africa as well as much of Asia (Karabatsos, 1985). In East Asia it occurs in Japan to 38°N (Tanaka and Inaba, 1986), China as far north as 44°N (Bai Wenbin, 1993) and Taiwan (Chiu and Lu, 1986). The vectors of BEF are less clearly known, but include both mosquitoes and *Culicoides* spp. In Australia BEF virus has been isolated from *C. brevitarsis* and *Anopheles bancrofti*, while Culex annulirostris is suspected but not proven as a major vector species (Muller and Standfast, 1986). Vector species have not been identified in East or South East Asia.

Simbu group viruses of the Bunyaviridae are also widely distributed in East Asia and the Western Pacific, in that group members AKA and Aino are known from both Japan and Australia. In Japan both these viruses have been isolated from mosquitoes including *Culex tritaenirhynchus* (Karabatsos, 1985) and from *C. oxystoma* (Kurogi et al., 1987), while in Australia they have been isolated from *C. brevitarsis* (Muller et al., 1982). Elsewhere other *Culicoides* spp. known as BLU vectors have also been implicated as AKA vectors, *C. variipennis* experimentally and *C. imicola* by isolation of virus from wild caught insects in the Middle East (Jennings and Mellor, 1989). Although *C. brevitarsis* has been identified in the southern islands of Japan (Wirth and Hubert, 1989), both these viruses and their vectors are largely unstudied between the northern and southern ends of their range in the Western Pacific.

These examples show that many viruses under study in Indonesia or Australia are also distributed in East Asia. In Japan, where more information has been published, there has also been interest in the possible regional redistribution of viruses by the dispersal of infected vectors by wind. Temporal associations between outbreaks of BEF in South Korea and Kyushu have occurred and a further possible association with BEF outbreaks in China noted. Upper air movements at the time were such to lead to the hypothesis that infected

Table 2
Bluetongue Viral Serotypes Isolated in the South East Asian–Australian Region

	BLU Serotype										
Country	1	2	3	7	9	12	15	16	20	21	23
Malaysia	+	+	+		+			+			+
Indonesia	+			+	+	+				+	+
Australia	+		+		+		+	+	+	+	+

vectors had been carried from the north-west to the south east, spreading the disease throughout the region (Ogawa, 1992; Shirakawa et al., 1994). However tropical storms moving from south-west to north-east have also been suspected of carrying insects infected with BEF and EHD 2 (Ibaraki) viruses from China to Japan at different times (Sellers, 1980).

Wind maps accompanying the report of Ogawa (1992) indicated that these same air movements would ultimately pass over Taiwan and southern China and the islands of South East Asia. Hence there may be a potential pathway for seeding of arboviruses into South East Asia, although at other times of the year the air movements are in the opposite direction, in the form of the south east monsoons (Anon., 1986). From a regional perspective it would be of interest to not only compare the arboviruses of western and eastern Indonesia, but also to compare each of these with viruses from the Philippines, where the dominant airstreams include not only the north east monsoons but also the south west monsoons (Flores and Balagot, 1969), resulting in that country being possibly exposed to influences from both western Indonesia and East Asia. Sellers (1980) described the potential for virus infected vectors to be moved in opposite directions by influences associated with the different seasons within the inter-tropical convergence zone.

Future studies of the relationships of viruses in different geographical areas, and their presumed movements, should employ molecular approaches. These techniques are also used in medical arbovirology, where the dengue viral serotypes have been topotyped (Trent, 1990). Hence for each of the 4 DEN serotypes, isolates from Thailand, the Philippines and Indonesia have been designated as having separate topotypes. For each serotype there are also other topotypes, up to a total of 10 for DEN 2, associated with other parts of Asia, Africa and the Americas. As noted by Gorman et al. (1981) for the BLU viruses, to account for this type of stable genetic difference among isolates within serotypes, associated with geographic location, there must have been prolonged periods of separation of viral populations to allow evolution in isolation.

The limited data available from observations on several different arboviral groups point to apparently opposite influences, molecular evidence for separate populations of viruses stable over time and, conversely, other evidence for the movement of viruses between geographic locations through the dispersal of infected vectors and changing mammalian host populations. There is much to be learned about the epidemiology of the important arboviruses of livestock affecting neighbouring countries in the region, with the essential first steps being the basics of isolation and characterisation of the viruses present, seroepidemiological studies and identification of the vectors.

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