Bluetongue, African Horse Sickness, *and* Related Orbiviruses

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EPIDEMIOLOGIC STUDIES OF BLUETONGUE VIRAL INFECTIONS IN INDONESIAN LIVESTOCK

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I. SUMMARY

Clinical BLU disease has not yet been diagnosed in Indonesian ruminants, although sheep imported from Australia have suffered severe mortalities with clinical signs and pathology consistent with BLU. Seroepidemiologic studies have shown that BLU virus infection are widespread throughout the islands of Indonesia, affecting buffaloes, cattle, sheep, and goats. Livestock at higher altitudes have a lower prevalence of infections than those in low altitude areas. Hence, the absence of clinical diseases is in spite of the presence of large populations of naive animals. Studies with sentinel groups of animals are showing that there is a seasonal pattern of infections with BLU group viruses, with a pattern of viral activity at the end of the wet season emerging. Confirmed isolations of BLU viruses in Indonesia are allowing more precise seroepidemiologic studies to be conducted, in which the geographic distribution and species susceptibility of the isolated serotypes are being determined.

II. INTRODUCTION

Serologic data indicate that BLU viral infections are widespread in Indonesia. A nation-wide survey of indigenous ruminants showed an overall 49% prevalence of reactors in the AGID test, varying from 26% in sheep to 74% in buffaloes. Reactors were found in each province tested.[1,2] AGID reactors were tested in SN tests to serotypes BLU-1, BLU-12, BLU-17, BLU-20, and BLU-21. Reactors to BLU-20 were most prevalent (23%), followed by BLU-21 (7%). Large ruminants reacted to all serotypes tested, but sheep to BLU-20 only.[3,4]

However, the testing of randomly collected sera can not describe the seasonal pattern of infections. Sentinel herd systems give more information on epidemiologic aspects, and also give opportunities for isolating viruses.[5] Since 1987, sentinel groups of cattle have been monitored in West Java, and recently from sites in eastern Indonesia. This paper

describes aspects of the program and presents preliminary results.

III. MATERIALS AND METHODS

A. SENTINEL COLLECTIONS

1. Collection Procedures

Sentinel groups of cattle usually comprised 10 to 15 animals aged 3 weeks to 5 months at the time of first sampling. Where possible, groups were started during the dry season, so maternal antibodies would have declined to undetectable levels by the start of the wet season, when arboviral activity was expected. Collections were made weekly or monthly for a one year period from individually identified cattle, after which the sentinel animals were changed. For weekly sampling, sera and heparinized blood were collected. Monthly collections comprised only sera. Sera were entered into a serum bank and held at -20°C until testing. Heparinized bloods were stored in duplicate in liquid nitrogen, or tested after holding for short periods at 4°C.

2. Sentinel Groups

a. West Java

Collections were made from Holstein cattle from 1987 until the present. At Depok, a low altitude site (30 m) with a six month wet and a six month drier season, and an annual rainfall of 2,500 mm, collections were made weekly. At Cisarua (altitude 1,300 m, rainfall 3,500 mm), also with six month wet and drier seasons, collections were made monthly.

b. West Timor

Collections were made weekly from Bali cattle from 1988 until the present, at Kupang (altitude 50 m, rainfall 1,250 mm), with a four month wet and an eight month drier season.

c. Irian Jaya

Collections were made weekly from two sites from 1989 until the present. Bali cattle were sampled at Jayapura (altitude 30 m, rainfall 2,750 mm), without clearly delineated wet and drier seasons. At Merauke (altitude 30 m, rainfall 1,750 mm), with a four month wet and an eight month dry season, collections were made from *Bos indicus* cattle.

B. SEROLOGICAL TESTS

1. AGID Test

All sera were screened in an AGID test.[2] BLU-20 (CSIRO 19) was used as antigen for West Java specimens from 1987 to 1989. For sera from Irian Jaya, Timor, and West Java from 1989 to 1990, BLU-1 (CSIRO 156) was the antigen.

2. SN Test

Sera from the Balitvet serum bank were tested at a dilution of 1:20 in an SN test[4] against Indonesian isolates (BLU-7 and BLU-9).[6]

C. VIRAL ISOLATIONS FROM BLOOD SAMPLES

Heparinized bloods from 1987 and 1988 were inoculated into BHK-21 cell cultures. Samples from 1989 and 1990 were diluted 1:10 in PBS and inoculated intravenously into 11 day-old ECE, and observed for 5 days for embryo lethal effect (ELE). Dead embryos at the first day post-inoculation (PI) were discarded, while from those with ELE from days 2 to 5 PI, the hearts were homogenized in media, filtered through 450 nm, and inoculated into C6/36 cell cultures. All C6/36 cultures were passaged in BHK-21 cells, which were discontinued if not showing CPE after 3 passages. CPE in BHK-21 cultures was considered to indicate a viral isolate.[7]

Isolates were stored at -70°C or in liquid nitrogen, and also used to prepare antigen for the AGID test and cover slips for the indirect IF (IIF) test for identification to viral group.[8] Serotype reactions using the SN test were then investigated, with confirmation and further serotyping being carried out by the World Reference Center for Bluetongue at Pirbright, UK.

IV. RESULTS AND DISCUSSION

This paper describes certain key components of the BLU research program in Indonesia, emphasizing serologic and virologic studies associated with weekly monitoring of sentinel groups of cattle, the species routinely showing a high reactor rate.[1-4] Some early results have been presented previously.[8] The first site at Depok has now been monitored for four years, and study sites in eastern Indonesia have been included. Where weekly blood sampling was practiced, weekly collections of insects were also made by light traps into alcohol for identification of potential vector species. At Depok, insect collections are also made into PBS for isolation of viruses. Results of insect collections will not be presented at this time. Since the sentinel herd scheme has been established, clinical cases of BLU have not yet been reported.

The patterns of seroconversions to BLU viruses in the AGID test are presented in Tables 1 and 2. As reported previously, at Depok in West Java seroconversions tended to be more frequent in the months of February and March when the rainfall reduces after a heavy wet season.[8,9] This picture is somewhat confused by a substantial percentage of seroconversions in July, August, and September, the three months which are historically the driest. Actual rainfall data have yet to be obtained.

Seroconversions were less frequent at the high altitude site at Cisarua, but again tended to be concentrated in the period April and May when historical rainfall records show the end of the wet season.[9] A prolonged, heavy, wet season may interfere with vector activity, and humid conditions at the end of the wet season or after rain may favor vector activity. However seroconversions have occurred at other times, such as June, the driest month, and January, the wettest month. Local factors, including unseasonal rain, may have to be considered. For instance, the cattle husbandry is more hygienic at Cisarua, where dung is spread on crops every few days rather than being left to pile up as at Depok. Such factors will affect breeding sites for Culicoides spp, the vector.

TABLE 1
The Percentage of Sentinel Cattle Seroconverting[a] in the BLU AGID Test at Two Sites in West Java, in Relation to Rainfall

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Depok (low altitude, high rainfall)												
1987	-	-	-	-	-	-	0	0	0	0	0	0
1988	0	0	75	0	8	9	0	-	-	-	-	-
1989	17	42	15	15	8	8	46	-	-	-	-	-
1990	0	-	75	0	0	17	0	44	33	11	-	-
RF[b]	358	318	282	265	200	140	87	79	58	134	285	284
Cisarua (high altitude, high rainfall)												
1987	-	-	-	-	-	-	0	0	0	0	0	0
1988	0	0	0	0	24	12	-	-	-	-	-	-
1989	0	14	0	14	28	0	0	0	-	-	-	-
1990	18	-	-	0	0	13	0	36	15	0	0	-
RF	702	470	517	339	157	93	112	146	142	209	233	399

[a] Seroconversion = an increase of two in the strength of reaction in the AGID test.
[b] RF = Average monthly rainfall in mm, based on historical records.[9]

In Timor where the average annual rainfall is only 1,000 to 1,500 mm,[10] the incidence of seroconversions has been much lower than at Depok, and more evenly spaced throughout the

year. Again, local factors may be as important as overall rainfall in the epidemiology of infections.

Little data is yet available from sites in Irian Jaya, and not all collections have been tested. Seroconversions are more prevalent in the higher rainfall area, Jayapura. A substantial percentage seroconverted in April, one of the wettest months, but also following a month in which the rainfall drops below 300 mm[10] as seen at Depok and Cisarua. At other times the monthly rainfall usually does not fall below 100 to 150 mm, and seroconversions have been observed in most months. At Merauke, the monthly rainfall is less than 100 mm for six months of the year, but seroconversions have been observed in the middle of the dry season, August, as well as in the wet season. Monitoring of these sites for several more years is necessary to determine accurate patterns.

The first year of weekly monitoring of sentinel cattle, 1987-1988, yielded the first isolates of presumed arboviruses,[8] two of which have been confirmed as BLU-7 and BLU-9,[6] and one of which has been identified as an EHD virus isolate, as yet untyped (unpublished). Table 3 shows the isolations of viruses to the present time, according to month of collection of the blood specimen. Comparing these data with the seroconversions to BLU viruses in the AGID tests for Depok in the years 1988 and 1990, a loose correlation can be seen. In Jayapura, seroconversions and isolations have occurred throughout the year.

TABLE 2
The Percentage of Sentinel Cattle Seroconverting[a] in the BLU AGID Test at Sites in West Timor and Irian Jaya, in Relation to Rainfall

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
West Timor - Kupang (low altitude, low rainfall)												
1988	-	-	-	29	0	0	0	23	13	0	17	13
1989	26	13	-	-	-	-	-	-	-	14	-	7
1990	13	0	0	10	0	25	-	-	-	-	-	-
RF[b]	350	350	250	75	12	12	12	12	12	12	75	250
Irian Jaya - Merauke (low altitude, intermediate rainfall)												
1989	-	-	-	-	-	-	-	-	-	-	-	-
1990	-	0	-	10	0	0	0	14	-	-	-	-
RF	250	250	250	175	125	37	12	12	12	37	75	175
Irian Jaya - Jayapura (low altitude, high rainfall)												
1989	-	-	-	-	-	-	-	-	-	0	33	13
1990	20	25	20	56	13	27	8	27	-	-	-	-
RF	350	350	250	350	175	175	175	175	125	175	175	250

[a] Seroconversion = an increase of two in the strength of reaction in the AGID test.

[b] RF = Average monthly rainfall in mm, based on historical records.[10]

TABLE 3
Preliminary Overview of Isolations of Presumed Arboviruses by Month

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Depok												
1988	-	1	2	1	1	-	-	-	-	-	1	0
1989	-	-	-	-	-	-	1	-	-	-	-	2
1990	4	5	4	11	5	5	-	-	-	-	-	-
1991	-	-	1	-	-	-	-	-	-	-	-	-
Jayapura												
1989	-	-	-	-	-	-	-	-	1	3	10	8
1990	6	2	-	3	1	1	1	-	-	-	-	-
Kupang												
1988	1	3	1	-	-	-	-	-	-	-	-	-
1990	-	-	-	1	-	-	-	-	-	-	-	-

To the present time, 86 suspected isolates have been obtained from 2028 samples processed. The yield may be increased by processing all inoculated ECE through cell cultures.[7] Reactions in the AGID and IIF tests place 14 isolates in the BLU or EHD virus groups, but not all have been tested. Six more of these, in addition to those already confirmed,[6] have been submitted to Pirbright for confirmation and serotyping. Indonesia has not yet imported a full range of BLU virus serotypes to allow this work to be done in the country itself.

Results of a preliminary survey for SN antibodies to BLU-7 and BLU-9 are presented in Table 4. Most sera were collected specifically for arboviral survey purposes in batches of 25 to 30 from certain villages, giving a 95% probability of detecting the presence of reactors with a 10% prevalence at the sampling location.[11] Hence the design of the survey could be considered a stratified random sampling. Since only a few locations in each of the tested provinces have been sampled to date, an apparently anomalous result is reported, that of no reactors in West Java, the province where the viruses were isolated. The sampling sites in this small survey were distant from the point of isolation of the viruses.

The overall prevalence results are not immediately comparable with the previous broad serologic survey of reactors to BLU-1, BLU-12, BLU-17, BLU-20, and BLU-21,[4] in which only group reactors in the AGID test were examined by SN, and the sera were tested at a dilution of 1:4 rather than 1:20. Nonetheless the sera in the current survey are drawn from all of the major islands from the west to the east of Indonesia, from locations where arboviral activity might be expected. The low overall reactor rate may indicate that these

TABLE 4
A Serologic Survey of Adult Cattle from Several Provinces in Indonesia for SN Antibodies against BLU-7 and BLU-9 Isolated in West Java

Province	BLU-7	BLU-9
North Sumatra	1/27	0/27
Lampung	0/69	0/69
West Java	0/118	0/118
Central Java	1/31	0/31
East Java	0/32	0/32
Bali	0/50	0/50
NTB (Lombok)	0/140	6/140
NTT (Timor)	6/192	30/192
South Kalimantan	0/95	0/95
South Sulawesi	0/25	0/25
Irian Jaya	0/134	6/134
Nation Wide Prevalence	8/913 (0.9%)	42/913 (4.6%)

two serotypes are not prevalent in Indonesian livestock. However, based on the sampling strategy, it appears that BLU-7 is distributed from North Sumatra through West and Central Java to Timor, a wide range. Present data indicate BLU-9 is found mainly in eastern Indonesia. Further studies are necessary to determine their full geographic ranges, and an analysis of sera collected from sentinel cattle is needed to determine their pattern of seasonal occurrence in provinces where their presence can be suspected on serologic grounds.

V. CONCLUSIONS

Although much has been accomplished, with useful and ongoing sampling procedures established in several areas, the elucidation of the BLU story in Indonesia is still in its early stages. The full range of serotypes likely to be present, their seasonality, and their distribution are not yet determined. Similarly, the species susceptibility to infection of both hosts and potential vectors is unknown and only preliminary work has been done on the geographic and seasonal distribution of vector species. The pathogenicity of isolates to local and imported breeds of livestock has not been studied. At the molecular level, it will be interesting to determine the relationships between Indonesian isolates and those of other countries, both within the region and around the world.

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