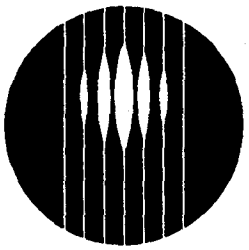

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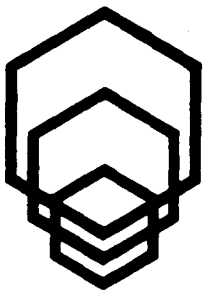

Q.I.M.R.

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LONGITUDINAL STUDIES OF *CULICOIDES* ASSOCIATED WITH LIVESTOCK IN INDONESIA

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INTRODUCTION

Culicoides spp, or the biting midges, belong to the family Ceratopogonidae, and include many important medical and veterinary pests biting man and animals. Some species are vectors of pathogens, especially of viruses of domestic livestock including bluetongue (BLU), African horse sickness (AHS), bovine ephemeral fever (BEF) and Akabane (AKA) viruses (Kettle 1984).

Very little has been reported from Indonesia on the biology of *Culicoides* spp and their involvement as viral vectors. BLU viruses have been isolated in Indonesia, with BLU serotypes 7 and 9 recovered from blood samples from healthy sentinel cattle near Bogor, West Java (Sendow *et al* 1988), and BLU serotypes 1, 12, 21 and 23 having been confirmed subsequently (Sendow *et al* in press).

Some species of *Culicoides* have been proven to transmit BLU viruses in Australia (*C fulvus, C wadai, C actoni, and C brevitarsis*) and an additional 3 species (*C brevipalpis, C oxystoma, and C peregrinus*) were shown to support viral replication (Standfast *et al* 1985).

A first step in studying the role of *Culicoides* spp as viral vectors in Indonesia is to describe the species present, and to ascertain whether those known as vectors elsewhere in the region are prevalent. This paper reports species of *Culicoides* found in some areas of Indonesia, and preliminary observations of their seasonal abundance.

MATERIALS AND METHODS

Collection Sites

Collections were made adjacent to sentinel groups of cattle which were monitored separately for serology and virus isolation. These sentinel groups were at sites located strategically across the length of Indonesia, in West Java, Bali, Timor and Irian Jaya. In Bali, West Timor and Irian Jaya collections were made from September 1989 to June 1992. In West Java collections were from August 1991 until July 1992, but are continuing at all sites.

(a) West Java: Collections were made at Depok (6° 24' S, 106° 48' E), a low altitude site (95 M), with a 6-month wet and a 6-month drier season and an annual rainfall of 2500 mm (Anon 1974), and at Cisarua (6° 41' S, 106° 58' E) a high altitude site (1071 M), rainfall 3500 mm, also with a 6-month wet and a 6-month drier season. Sentinel cattle at each site were on small dairy farms of about 30 cattle. At Depok faeces was heaped close to the pens for long periods until spread on adjacent fields. Drainage was poor and the site has proved useful for insect collections.

(b) Bali: Collections were made near Denpasar (8° 40' S, 115° 14' E) at an altitude of 40 M and with an annual rainfall of 2000 mm, with a 5-month wet and a 7-month drier season.

(c) West Timor: Collections were made near Kupang (10° 11' S, 123° 42' E) (altitude 50 M, rainfall 1250 mm) with a 4-month wet and an 8-month dry season.

(d) Irian Jaya: Collections were made from 2 sites, near Jayapura on the north coast (2° 41' S, 140° 52' E) (altitude 30 M rainfall 2750 mm) without clearly delineated wet and dry seasons, and Merauke (8° 16'S, 140° 13' E) on the south coast (altitude 30 M, rainfall 1750 mm) with a 4-month wet and an 8-month dry season.

Collection Procedures

In Bali, West Timor and Irian Jaya battery powered modified CDC light traps (Dyce *et al* 1972) were used, sited adjacent to livestock, particularly penned cattle, and also adjacent to standing surface water where possible. Traps were set in the late afternoon and allowed to run until daybreak. The catches were held in 70% alcohol.

In West Java, insects were collected from August 1991 to July 1992 with a Pirbright-type miniature light trap operated from the mains supply via a 12 volt step-down transformer. Collections were made from 4.30 pm to 8.00 pm once a week. In this trap, insects were not collected into alcohol, but were blown directly into a bottle containing phosphate buffered saline (PBS) supplemented with antibiotics and containing 0.1% detergent, since virus isolation was to be attempted.

The weekly catches from each location were sorted in the laboratory under a binocular micro-

TABLE 1

Culicoides species collected at sentinel cattle sites in Indonesia.

Culicoides Species	Locations					
	Jayapura	Merauke	Kupang	Denpasar	Depok	Cisarua
Subgenus *Avaritia*						
actoni	*	*	–	–	*	*
brevipalpis	–	–	*	–	–	–
brevitarsis	*	*	*	*	*	*
dumdumi	*	–	–	–	–	–
flavipunctatus	*	*	*	*	*	*
fulvus	*	*	*	*	*	*
jacobsoni	*	*	–	–	*	*
maculatus	*	*	–	–	*	*
nudipalpis	*	*	*	*	–	–
orientalis	*	*	*	*	*	*
pungens	–	*	–	–	–	–
wadai	*	*	*	–	*	*
Subgenus *Haemophoructus*						
gemellus	*	*	–	–	*	*
Subgenus *Hoffmania*						
effusus	*	–	*	*	–	–
insignipennis	–	–	–	–	*	*
liui	–	–	–	–	–	*
parabubalus	*	–	–	–	–	–
peregrinus	*	*	*	*	*	*
sumatrae	*	*	*	–	*	*
Subgenus *Trithecoides*						
albibasis	–	–	*	–	*	–
anophelis	–	–	–	*	*	*
barnetti	*	–	*	*	*	*
flavescens	–	–	*	–	–	–
gewertzi	*	–	*	–	*	*
huberti	*	–	–	–	–	–
palpifer	*	–	*	*	*	*
parabarnetti	*	–	–	–	–	–
parahumeralis	–	–	*	*	*	*
Subgenus *Meijerehelea*						
arakawae	*	–	*	*	*	–
guttifer	*	–	*	*	*	*
histrio	*	*	*	–	–	–
Other						
ardleyi	*	–	–	–	–	–
austropalpalis	–	*	–	–	–	–
cameronensis	–	–	–	–	*	*
clavipalpis	–	–	–	*	–	–
geminus	*	–	*	*	*	*
huffi	–	–	–	*	*	–
neomelanesia	*	–	–	–	–	–
ornatus	–	*	–	–	–	–
pampangensis	*	–	–	–	–	–
oxystoma	*	*	*	*	*	*
pangkorensis	–	*	*	–	–	–
papuensis	–	–	*	–	–	–
pelilioensis	–	*	*	–	–	–
petersi	*	–	–	–	–	–
pseudostigmatus	*	*	–	–	–	–
pygmaeus	*	–	–	–	–	–
semicircum	*	*	–	*	–	–
shortti	–	–	–	–	*	*
Total	32	21	24	19	24	22

scope. From catches in West Java, the female *Culicoides* were sorted to species and the parous females were separated on the basis of abdominal pigmentation. Parous adults were distinguished by the development of a dark red pigment in the epidermal or subepidermal layers of the abdomen (Dyce 1969).

Identification of *Culicoides* was based on standard texts (Dyce, unpublished; Ratanaworabhan 1975; Tokunaga 1959; Wirth and Hubert 1989).

Numbers of each species from every trapping were counted. Insects that were difficult to identify were mounted for independent confirmation by Mr Alan L Dyce. Mounted specimens were first prepared by serial washing in alcohol (70%, 80%, 90% and absolute) followed by clearing in creosote. They were mounted in thin xylol-balsam.

RESULTS

Distribution of *Culicoides* spp

From 6 trapping locations 49 species of *Culicoides* have been collected. *C brevitarsis, C fulvus, C flavipunctatus, C orientalis, C peregrinus* and *C oxystoma* were found at all sentinel sites.

TABLE 2

Numbers of the ten most abundant species in light traps at 2 sentinel cattle sites in West Java (Depok and Cisarua) from August 1991 to July 1992.

Species	Depok		Cisarua	
C actoni	648	(5)*		
C barnetti	444	(7)	228	(3)
C fulvus	1,226	(3)	304	(2)
C jacobsoni			64	(10)
C maculatus			131	(6)
C orientalis	199	(9)	163	(5)
C oxystoma	1,302	(2)	93	(7)
C palpifer	287	(8)	92	(8)
C parahumeralis	645	(6)	748	(1)
C peregrinus	1,193	(4)	65	(9)
C shortti	163	(10)		
C sumatrae	3,251	(1)	200	(4)

* Rank order of abundance

(a) West Java: Culicoides found at sentinel cattle sites in Depok and Cisarua are listed in Table 1. *Culicoides* collected at Depok included 24 species and at Cisarua 22. In Depok, *C sumatrae* was the dominant species, followed in decreasing order of abundance by *C oxystoma, C fulvus, C peregrinus, C actoni* and others (Table 2). In Cisarua, *C parahumeralis* was the dominant species, followed by *C fulvus* (Table 2).

More *Culicoides* were collected at Depok than at Cisarua. For a year (August 1991 to July 1992) at Depok, 10,021 *Culicoides* were trapped while at Cisarua 2249 were caught. The highest monthly total of *Culicoides* at Depok was 2949 while at Cisarua it was 684.

(b) Bali: At Denpasar 19 species were collected (Table 1). *C peregrinus* was by far the most abundant species, followed by much fewer numbers of *C oxystoma* (Table 3).

TABLE 3

Numbers of the ten most abundant species in light trap collections at each of 4 sentinel cattle sites in Eastern Indonesia (Jayapura, Merauke, Kupang, Denpasar) from September 1989 to June 1992.

Culicoides Species	Numbers of Species			
	Jayapura	Merauke	Kupang	Denpasar
albibasis				39 (5)*
arakawae			208 (8)	176 (3)
barnetti	34 (9)		333 (6)	126 (4)
brevitarsis	2,453 (2)	537 (2)	2,521 (2)	
effusus	36 (8)			
flavipunctatus		49 (3)		10 (10)
fulvus	139 (4)	12 (7)	258 (7)	
geminus			1,429 (5)	
guttifer	59 (6)			
histrio		47 (5)	2,434 (3)	
huberti				14 (9)
huffi				38 (6)
nudipalpis	62 (5)	4 (9)	122 (10)	
orientalis	24 (10)	48 (4)		
ornatus		18 (6)		
oxystoma	459 (3)	4 (8)	9,234 (1)	312 (2)
palpifer				29 (7)
parahumeralis			137 (9)	28 (8)
peregrinus	85,739 (1)	2,788 (1)	1,628 (4)	4,268 (1)
wadai	38 (7)	4 (10)		
Other spp	54	18	530	58
Total	89,097	3,929	18,834	5,040

* Rank order of abundance

(c) West Timor: In Kupang 24 species were collected (Table 1). *C oxystoma* predominated, with *C brevitarsis, C histrio, C peregrinus* and *C geminus* also being well represented in collections (Table 3).

(d) Irian Jaya: At Jayapura 32 species of *Culicoides* were collected at the sentinel cattle site (Table 1). In this location *C peregrinus* was the most abundant species while *C brevitarsis* was the second most abundant (Table 3).

During this survey in Merauke, *C peregrinus* was the most abundant species among a total of 22 species in the collections (Table 1), followed again by *C brevitarsis* (Table 3).

Seasonal Fluctuations

The monthly totals of *Culicoides* spp collected in light traps at Depok and Cisarua in West Java are presented in Figures 1 and 2. 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. From Figure 3 it can be seen that the increase in numbers in September was associated with an increase in *C oxystoma*, that numbers of *C peregrinus* increased slightly in January, and that April saw a proliferation of *Avaritia* spp.

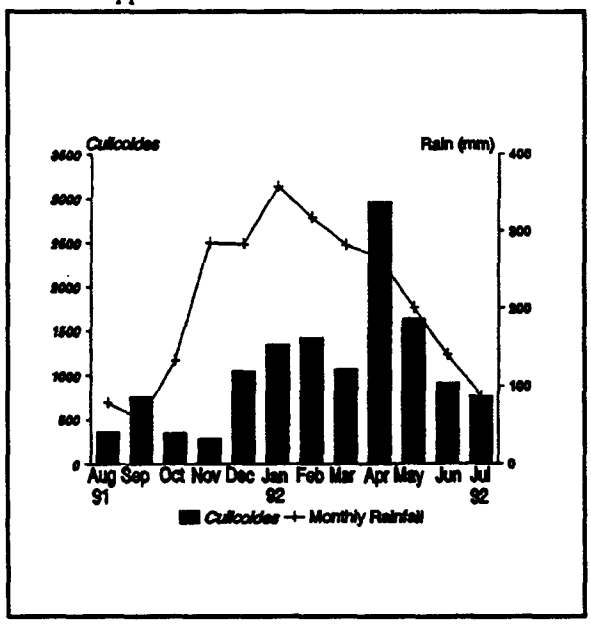

Figure 1. Total *Culicoides* at Depok, 1991–1992.

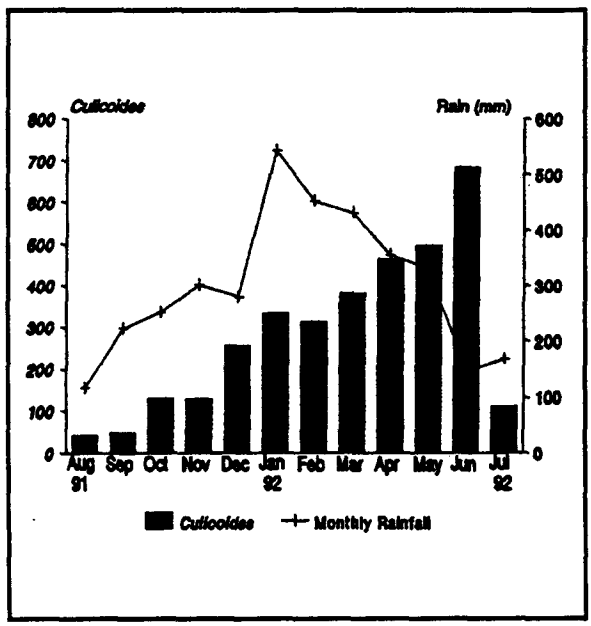

Figure 2. Total *Culicoides* at Cisarua, 1991–1992.

At Cisarua (Fig 2) numbers increased steadily until June, then fell sharply. There was a small increase in numbers of *C oxystoma* in October, of *C peregrinus* in December, and a substantial increase in May and June (Fig 4).

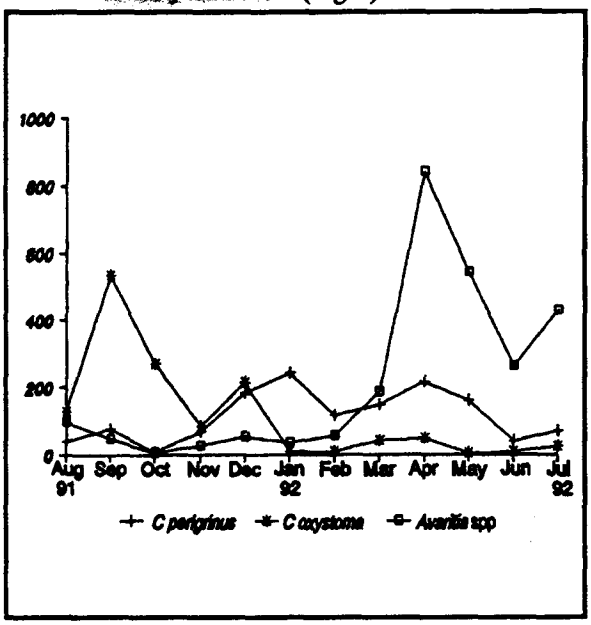

Figure 3. *C peregrinus*, *C oxystoma* and *Avaritia* spp at Depok, 1991–1992.

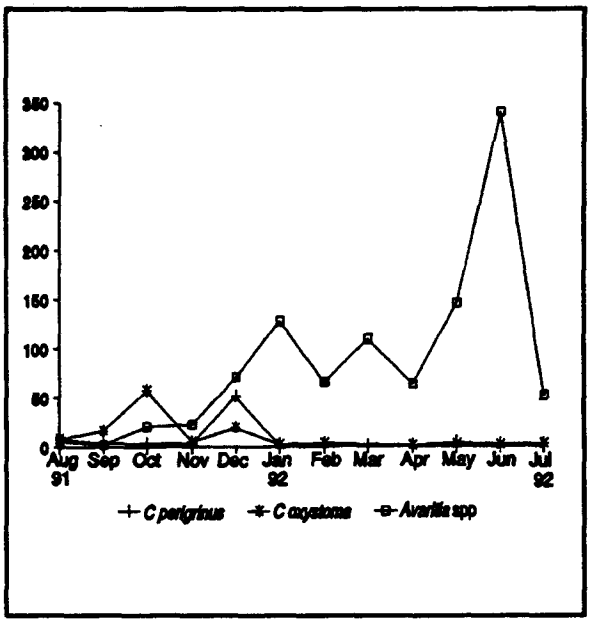

Figure 4. *C peregrinus*, *C oxystoma* and *Avaritia* spp at Cisarua, 1991–1992.

The total numbers of *Culicoides* trapped at Denpasar and in Eastern Indonesia in 2 successive years are presented in Figures 5 to 8.

There was year-to-year variation as well as variation with each site. In Denpasar, peak numbers were observed in February/March in each year (Fig 5), while in Kupang peak numbers occurred in October in 1989/90 and in December in 1990/1991, with smaller peaks throughout each year (Fig 6). In Merauke peak numbers were also reached earlier in the first year then in the second (Fig 7). In Jayapura the main activity was in June/July, with large catches also occurring in other months (Fig 8).

The patterns of seasonal abundance of the *Avaritia* subgenus, *C oxystoma* and *C peregrinus*

have also been calculated for the sentinel sites in Eastern Indonesia over 2 successive years. However, as space precludes the presentation of a graph for each site for each year, the information has been condensed in Table 4.

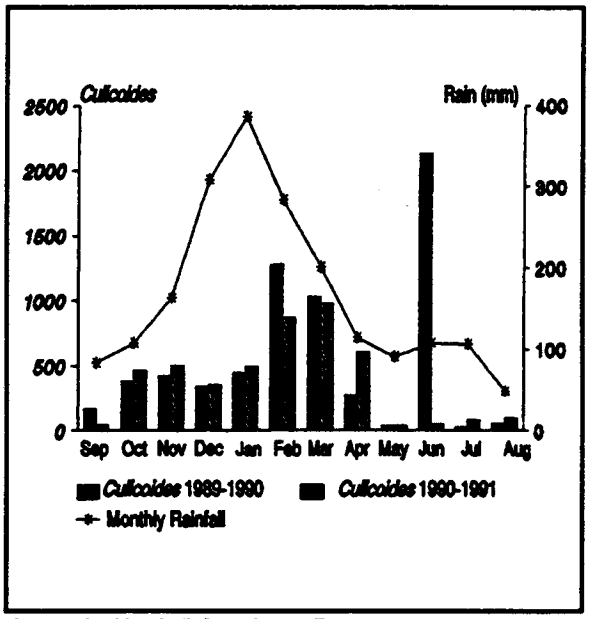

Figure 5. Total *Culicoides* at Denpasar, 1989–1991.

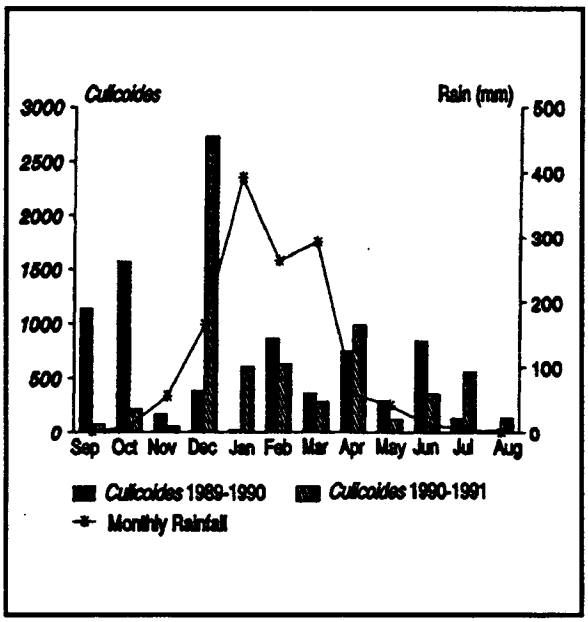

Figure 6. Total *Culicoides* at Kupang, 1989–1991.

DISCUSSION

The primary purpose of the present study is to identify the *Culicoides* that may be functioning as viral vectors, especially of BLU viruses. There are many species of *Culicoides*, and these have been subdivided into subgenera together with a large number of more loosely grouped species (Wirth and Hubert 1989). Elsewhere in the world the main proven vectors of BLU are predominantly from the subgenus *Avaritia*: *C actoni, C brevitarsis, C fulvus* and *C wadai* in Australia and *C imicola* in Africa (Standfast *et al* 1985). In Central America the main vector is *C insignis* from the subgenus Hoffmania (Greiner *et al* 1985), while in North America the main vector is *C variipennis* of the subgenus *Monoculicoides*. There do not appear to be members of this latter subgenus in South East Asia (Wirth and Hubert 1989), but members of the other major groups were represented in collections.

TABLE 4

Months of peak catches of *C oxystoma, C peregrinus* and the subgenus *Avaritia* in light traps at Denpasar and in Eastern Indonesia.

Species/Site	Month			
	1989/1990		1990/1991	
C oxystoma				
Denpasar	Oct	(57)	Oct	(24)
Kupang	Sep	(1,266)	Dec	(1,351)
Merauke	–	(4)	–	(4)
Jayapura	Oct	(303)	Dec	(162)
			Jun	(182)
C peregrinus				
Denpasar	Feb	(1,259)	Mar	(450)
Kupang	Oct	(211)	Dec	(349)
Merauke	Nov	(685)	Feb	(292)
Jayapura	Dec	(2,597)	Oct	(3,969)
	Jul	(6,759)	Jun	(5,745)
Avaritia				
Denpasar	Oct	(7)	Oct	(7)
Kupang	Feb	(529)	Dec	(1,006)
Merauke	Nov	(42)	Feb	(282)
Jayapura	Dec	(251)	Jun	(478)

Table 1 shows that members of the subgenus *Avaritia* were present at all sites, especially *C brevitarsis* and *C fulvus*, proven to be vectors in Australia. In Eastern Indonesia, in Jayapura, Merauke and Kupang, *C brevitarsis* was the second among the 10 most abundant species, with *C fulvus* also among the 10 at each site. Hence there is the potential for BLU and related viruses to be spread in those areas. In Bali, members of the *Avaritia* did not feature significantly in collections, while in West Java, although *C brevitarsis* was not collected in any numbers, *C fulvus* was the second or third most abundant species, and other *Avaritia* such as *C actoni, C jacobsoni, C maculatus* and *C orientalis* were all among the 10 most abundant species. The vector status of most of these latter species is unknown.

There is considerable interest in identifying vector species in Indonesia that have not been proven as vectors elsewhere. In laboratory studies in Australia, *C peregrinus, C oxystoma* and *C brevipalpis* were found to support replication of BLU after feeding on viraemic sheep (Standfast *et al* 1985). *C brevipalpis* in the subgenus *Avaritia* was found only at Kupang in West Timor.

C peregrinus is in the subgenus *Hoffmania* that also includes the Central American vector *C insignis*. It was present at all sites, and was frequently the most abundant midge caught. *C oxystoma* is a species not assigned to a subgenus that also occurred at all sites in considerable abundance, especially in Kupang and Denpasar. There is great interest in conducting further studies of these 2 species.

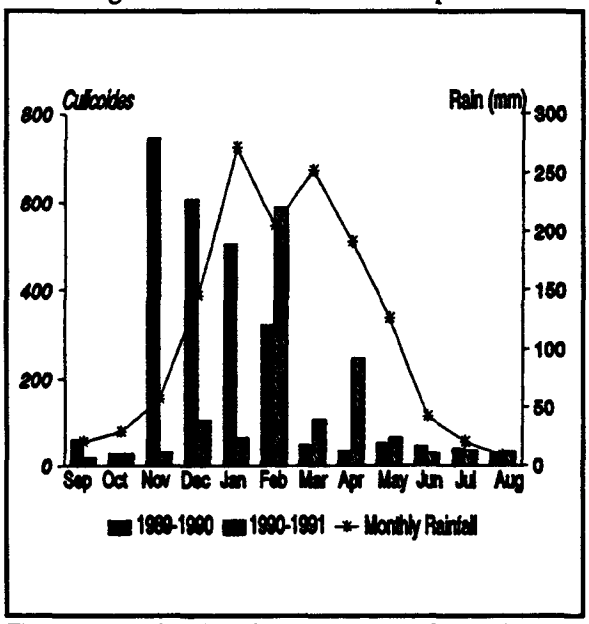

Figure 7. Total *Culicoides* at Merauke, 1989–1991.

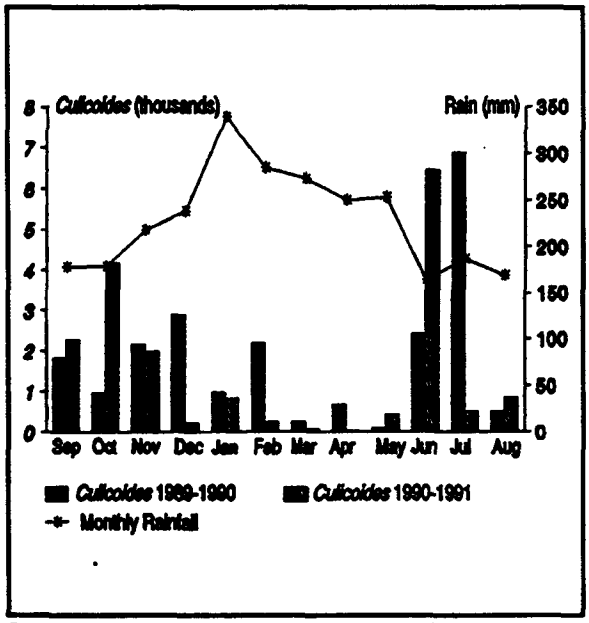

Figure 8. Total *Culicoides* at Jayapura, 1989–1991.

Two members of the *Avaritia* that feed on livestock but which are not present in Australia are *C orientalis* and *C nudipalpis* (Standfast and Muller 1989). *C orientalis* was collected at all 6 sites, while *C nudipalpis* was collected from all sites in eastern Indonesia, but not from the West Java sites. It is also of interest to determine if these species are vectors of any BLU serotype, for spread of these insects within the region may be associated with new risks. *C nudipalpis* was among the 10 most abundant species at Jayapura, Merauke and Kupang, while *C orientalis* was among the 10 most abundant at both the Irian Jaya and West Java sites, at opposite ends of the country. *C nudipalpis* may be of particular interest because it is nearly indistinguishable from *C imicola*, the major BLU vector in Africa, and occurs only in the islands of SE Asia, where *C imicola* is not present (Wirth and Hubert 1989).

Another member of the *Avaritia* that was widely distributed was *C flavipunctatus*, being one of the 6 species found at all 6 sites, though not present in relative abundance. *C wadai*, a vector of major concern in Australia, was among the 10 most collected species only in Irian Jaya.

Based on the above considerations, particular attention was paid to the seasonal abundance of potential BLU vectors, as assessed by their presence in light trap collections. Members of the subgenus *Avaritia* were considered as a group, with *C oxystoma* and *C peregrinus* being considered separately.

In West Java, each of the 3 groups showed a peak abundance at different times. In general, the peaks in collections were a month later at Cisarua than at Depok. This pattern has been observed previously for seroconversions to BLU group viruses (Sendow *et al* 1991). Historically, seroconversions to BLU in these study areas have occurred at the end of the wet season, at about the time that the *Avaritia* populations peaked in this year of observation (Sendow *et al* 1991). It remains to correlate these observations of insects with actual seroconversions and isolation of viruses from sentinel cattle in this year of study.

Acknowledgments

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