Anthropophilic mosquitoes at Richards Bay, Natal, and arbovirus antibodies in human residents

B. L. SHARP[1], C. C. APPLETON[1], D. L. THOMPSON[2] AND G. MEENEHAN[2]

[1]*Research Institute for Diseases in a Tropical Environment of the South African Medical Research Council, P.O. Box 17120, Congella 4013, Republic of South Africa;* [2]*Arbovirus Research Unit, National Institute for Virology, Private Bag X4, 2131 Sandringham, Johannesburg, Republic of South Africa*

Abstract

A survey at Richards Bay, the site of a new international harbour on the east coast of South Africa, revealed the presence of 11 anthropophilic mosquito species. These are discussed in relation to their known medical importance and to a complementary survey of arbovirus antibodies among residents of the area. *Mansonia uniformis* was by far the most abundant man-biting mosquito. Antibodies against Germiston, Banzi and Rift Valley Fever viruses were the three most commonly encountered, being detected in 29·7, 12·2 and 8·7% of residents respectively. This new knowledge updates the distribution and prevalence of arbovirus antibodies in man in northern Natal.

Introduction

Richards Bay (28°49'S ; 32°05'E) is the site of an international harbour on the eastern seaboard of the Republic of South Africa (RSA) (see Figure). The area surrounding the harbour site, the Umhlatuze delta, is an extensive low-lying wetland. Industrialization and town construction have greatly altered this wetland which, however, still experiences a serious mosquito nuisance problem (APPLETON & SHARP, 1985). The health authorities decided to investigate the mosquito species involved so that appropriate control measures could be implemented. There were also anecdotal reports suggesting that arthropod-borne virus transmission occurred in the area (S. M. Joubert and D. M. Eckard, personal communication).

We first investigated the use of swamp types within this wetland as mosquito breeding areas (APPLETON & SHARP, 1985). The present study ascertained which mosquito species were biting man, their potential medical importance and the background levels of antibodies to arthropod-borne viruses in the local human population.

Materials and Methods

Collection of anthropophilic mosquitoes

Data were collected between October 1981 and June 1982. Man-biting mosquitoes were caught in a man-baited net. Night catches commenced before last light and were accumulated as hourly totals; daylight catches were made from 0900h to 1600h. Mosquitoes were identified individually by morphology, the physiological technique of MUIR-HEAD-THOMPSON (1951) being used to confirm the identity of *Anopheles merus*.

Emergence trapping

Mosquitoes emerging from the swamps were sampled from October 1981 to January 1983, using the floating traps described by APPLETON & SHARP (1985). These authors typed the swamps within the townlands of Richards Bay according to the dominant aquatic vegetation present as listed below. (i) Reed swamps dominated by *Phragmites australis* and with a carpet of duckweek, *Lemna* sp., on the water surface. (ii) Grass swamps dominated by *Leersia hexandra*, the sedge *Cyperus isocladus* and the fern *Thelypteris totta*. (iii) Bulrush swamps dominated by *Typha latifolia*, the sedge *C. sensilis* and *T. totta*. (iv) Papyrus swamps dominated by *C. papyrus*. (v) Drained swamp forest dominated by the trees *Barringtonia racemosa* and *Hibiscus tiliaceus* and containing numerous isolated pools. (vi) Inundated swamp forest dominated by *B. racemosa* and with *Lemna* sp. floating on the surface and *Myriophyllum spicatum* beneath.

Daytime collection of resting mosquitoes

Daytime searches for resting mosquitoes were carried out in various sheltered sites within and around the residential area of Richards Bay. These included indigenous coastal and swamp forest as well as reed and papyrus swamps.

Serological survey

Five ml blood samples were collected from each of 96 children at Msingazi Primary School (6 km NE of Richards Bay) and from 172 adults in Richards Bay town itself in November and August 1982, respectively.

The children had all been born in Kwa-Mbonambi, 17·5 km from the school. The adults included 34 lifelong local residents and 138 others from many parts of the RSA who had resided in the area for more than 4 years. All 268 sera were tested for antibodies to 10 viruses by the haemagglutination inhibition test (HAI) described by SHOPE & SATHER (1979), who used 8 HA units of antigen.

Sera found positive (32 from adults and 12 from school-children) by the HAI test for antibodies to the flaviviruses Banzi (BAN), Wesselsbron (WSL) and West Nile (WN) were tested for neutralizing antibodies against these viruses by means of the mouse protection (neutralization; Nt) test (KOKERNOT *et al.*, 1956; MCINTOSH *et al.*, 1962); 12 baby mice, 1-3 days old, in 2 litters, were injected with 0·2 ml of each virus-serum mixture, survival of 6/12 or more indicating protection. Another group of 21 adult and 9 children's sera were tested for neutralizing antibodies to Pongola virus and, from these, 13 and 3 respectively for antibodies neutralizing Bwamba virus. Localities mentioned in the text are shown in the Fig.

Results and Discussion

Man-biting species

A total of 96·25 hours of outdoor man-baited net catches was made on 17 nights, and 12 hours of daylight man-baited net-catches on 2 days. Table 1 shows the 11 species of mosquito caught during this study in the man-baited net. These species are discussed in terms of their potential medical importance in order of relative abundance in our collections.

Mansonia uniformis, the predominant mosquito species collected using human bait, was 7·7 times

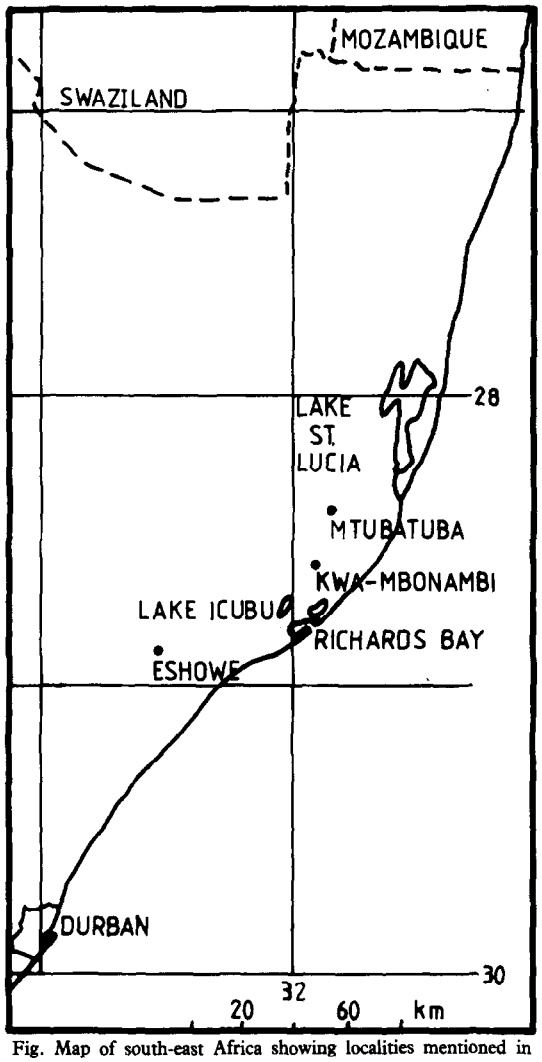

Fig. Map of south-east Africa showing localities mentioned in the text.

more abundant than *Culex theileri* the next most common species found. *M. uniformis* was consistently collected during the entire study period and man-biting rates of up to 162 per hour were recorded. This mosquito must therefore play the major role in the nuisance problem in the Richards Bay area. *M. uniformis* was also found breeding in all the swamp types except the swamp forest (APPLETON & SHARP, 1985). Adults were found resting in the coastal forest, reed swamps and swamp forests. Considering that these data were collected during a period when Natal was experiencing one of its worst droughts in recorded history (ERSKINE, 1983), the potential breeding area must have been reduced and one can only infer that the man-biting rates during normal years must be considerably higher. In Asia *M. uniformis* is known to transmit filariasis (WHARTON, 1962), a disease which does not occur in South Africa. Three arboviruses, Wesselsbron, Ndumu and Spondweni, have been isolated from this species in the RSA (WORTH *et al.*, 1962; MCINTOSH, 1975).

Table 1—Mosquito species caught in man-baited net catches

Species	Total	Mean/h	Range/h
Mansonia uniformis	2953	27·0	0-162
Culex theileri	334	3·5	0-50
Aedes durbanensis	194	2·0	0-30
Anopheles tenebrosus	112	1·1	0-16
Mansonia africana	103	1·0	0-15
An. merus	27	0·3	0-3
Culex thalassius	10	0·1	0-4
Coquilettidia sp.	9	0·1	0-2
Ae. aegypti	4	0·3	0-1
Eretmapodites quinquevittatus	2	0·16	0-1
Ae. strelitziae	2	0·16	0-2

Culex theileri was generally present in small numbers with the exception of one night in April 1982 when 178 were caught (53% of the number collected during the entire study period). This is the largest sample of this mosquito collected to date on the Natal Coast (P. G. Jupp, personal communication). This species was found to breed in reed swamps only. *C. theileri* is known to be a primary epizootic vector of Rift Valley Fever virus and a secondary vector of West Nile and Sindbis viruses (MCINTOSH, 1975).

Aedes durbanensis was found mainly during February 1982 when 59% of the total number were caught. This species was shown by emergence trapping to breed in the grass swamps and is not of known medical importance.

Anopheles tenebrosus was consistently caught in the man-baited net throughout the study period. Emergence trap data showed that it breeds in papyrus and reed swamps. The medical importance of *A. tenebrosus* is considered to be negligible (GILLES & DE MEILLION, 1968).

Mansonia africana was caught in small numbers in the man-baited net and according to emergence trap data it breeds in areas of inundated grass and reed swamps. This species has been implicated in the transmission of Chikungunya virus (PATERSON & MCINTOSH, 1964; MCINTOSH *et al.*, 1965; DE MOOR *et al.*, 1976).

An. merus occurred only in small numbers. This species has been shown to transmit filariasis in Kenya (BUSHROD, 1981) and malaria in Tanzania and Kenya (MUIRHEAD-THOMPSON, 1951; MOSHA & PETRARCA, 1983). The role of *An. merus* in malaria transmission in South Africa has not been investigated.

Culex thalassius, a species of unknown medical importance, was never caught in large numbers with human bait and its breeding area was not identified by means of emergence traps. However, in 1965 this species was an important nuisance mosquito biting man during a period when the harbour was kept open by dredging operations. Silt and seawater dumped on the grassed shore created ideal breeding places for *C. thalassius* (B.M. McIntosh, personal communication). The salinity of these breeding pools was 8·0 to 9·5 parts per thousand (J. Turner, unpublished data).

Coquilettidia spp. were trapped in small numbers in the man-baited net but large numbers were caught by emergence trapping and in daytime resting catches in swamps and coastal forest. Breeding areas used were reed, grass, papyrus and bulrush swamps. The species caught were *Coq. metallicus*, *Coq. aurites*, *Coq.*

aurea/wahlbergi, Coq. chrysosoma, Coq. annulata and *Coq. maculipennis*. This genus is considered to be of limited medical importance as it does not generally feed on man. West Nile virus has been isolated from *Coq. metallicus* but this was believed to involve bird-to-bird transmission; Usutu virus was isolated from *Coq. aurites* (GILLETT, 1972).

The daylight man-baited net catch rate was low ($\bar{x}$ = 0·16 mosquitoes/hour), perhaps due to a lack of suitable breeding sites as a consequence of the drought in the area at the time. These catches comprised three species. *Ae. aegypti* is a vector of yellow fever but the virus is not endemic in South Africa. *Ae. aegypti* was found to rest in the coastal forest. *Ae. strelitziae* and *Eretmapodites quinquevittatus* were caught in the man-baited net and in the coastal forest. Neither species is of any known medical importance.

Arbovirus antibody survey

Sera from the subjects examined were positive for antibodies against 8 arboviruses in adults and 4 in children (Table 2). This table contains the combined results of the HAI and Nt tests.

Of the 44 sera tested by the mouse protection test for neutralizing antibodies against BAN, WSL and WN viruses, only 30 were found positive.

Significant past infection rates for all adults sampled were shown for Banzi (12·2%), Germiston (29·7%) and Rift Valley Fever (RVF) (8·7%) viruses. Of the 34 adults who had lived in the Richards Bay area all their lives, 38·2% had antibodies against Germiston virus, 8·8% against Wesselsbron and 5·8% against Banzi, Witwatersrand and Rift Valley fever viruses. The children sampled showed 5·2, 4·2 and 2·1% with antibodies against Wesselsbron, Chikungunya and Banzi viruses respectively.

The vector of Banzi, Germiston and Witwatersrand viruses is *Culex rubinotus* (JUPP *et al.*, 1976; MCINTOSH *et al.*, 1976). This mosquito is present in the area studied and was caught in emergence traps and resting in reed swamps. It bit man freely during the crepuscular period in these habitats. The collection of 334 *C. theileri*, an epizootic vector of RVF, may have a bearing on the moderate level of subjects with antibodies to this virus.

SMITHBURN *et al.* (1959) collected blood samples from residents of 25 localities in Natal and tested them for antibodies to a number of arboviruses by the Nt test. The localities can be divided into those north of Mtubatuba (28°25'S; 32°10'E) and those to the south (including Mtubatuba), a division made on the basis of the more tropical nature of the northern areas (POYNTON, 1961). The results obtained by SMITHBURN *et al.* (1959) in lowland areas, and comparable with those of the present study, are given in Table 3. They reported antibodies to Sindbis virus to be absent (or rare) in areas south of Mtubatuba. This was confirmed by the present study in which no antibodies to Sindbis virus were detected in the sera of children, or of the lifelong resident adults sampled.

SMITHBURN *et al.* (1959) were unable to detect antibodies to Chikungunya virus in adults in the southern areas and they did not test any children in the south. However, 4·2% of the children examined during the present study were positive, higher than the 1·0% reported by SMITHBURN *et al.* (1959) for children in the north. This indicates that Chikun-

Table 2—The presence of antibodies to arboviruses in humans at Richards Bay

		Percentage of subjects positive for antibodies					
		Group 1		Group 2		Group 3	
Virus	*Test*	*No.*	%	*No.*	%	*No.*	%
Sindbis[1]	HAI	8	4·7	0	—	0	—
Chikungunya[1]	HAI	2	1·2	0	—	4	4·2
Banzi[2]	HAI + Nt	1+20[b]	12·2	2	5·8	2	2·1
Wesselsbron[2]	HAI + Nt	8	4·7	3	8·8	2+3	5·2
West Nile[2]	HAI + Nt	4+4[b]	4·6	0	—	0	—
Dengue 1[2]	HAI	0[c]	—	0	—	0	—
Dengue 2[2]	HAI	0	—	0	—	0	—
Germiston[3]	HAI	51	29·7	13	38·2	0	—
Rift Valley Fever[5]	HAI	15	8·7	2	5·8	0	—
Witwatersrand[4]	HAI	6	3·5	2	5·8	0	—
Pongola[3]	Nt	0	—	0	—	1	10·0
Bwamba[3]	Nt	0	—	0	—	0	—

b = represents monotypic HAI positives + Nt positives
c = 4 adult sera were positive for DEN 1 + 2 (1:20), regarded as cross-reactions.
Group 1 - sera from 172 adults that had lived in the area for longer than 4 years.
Group 2 - sera from 34 adults that had lived in the area all their lives.
Group 3 - Sera from 96 children that had lived in the area since birth.

1 = Alphaviruses
2 = Flaviviruses
3 = Bunyaviruses
4 = Bunyavirus-like
5 = Phleboivirus

Table 3—Comparison of arbovirus antibody levels found by SMITHBURN *et al.* (1959) and in the present study

Virus	Percentage of subjects positive for antibodies					
	North of Mtubatuba Smithburn		*South of Mtubatuba* Smithburn		Present study	
	Children/Adults		Children/Adults		Children/Adults[+]	
Sindbis	1·3	5·3	0·0	0·0	0·0	0·0
Chikungunya	1·0	13·3	NT	0·0	4·2	0·0
Rift Valley Fever	5·8	15·7	NT	0·0	0·0	5·8
Wesselsbron	14·5	44·7	1·3	13·2	5·2	8·8
Banzi*	13·3	24·2	6·9	15·2	2·1	5·8
West Nile	2·4	0·5	0·0	0·0	0·0	0·0

* Banzi = H336
NT = none tested
[+] Group 2, Table 2

gunya virus is endemic in the Richards Bay area, which is a southward extension of its known distribution (previously not known to occur south of the St Lucia Lake area (MCINTOSH, 1980). The established vector of Chikungunya virus is the *Ae. furcifer-taylori* group (MCINTOSH *et al.*, 1977). These mosquitoes were not caught during the present study. Lake St Lucia was also regarded as being their approximate southern limit. This species occurs only after rains in or near woodland and the fact that the present catches were made during a drought period may account for their absence from our samples. Alternatively, it is known from laboratory transmission experiments that this virus can be transmitted by *Ae. aegypti* and *Mansonia africana* (PATERSON & MCINTOSH, 1964; MCINTOSH *et al.*, 1965; JUPP *et al.*, 1981) and it is possible that these species are responsible for infections in the area. However, no antibodies to Chikungunya virus were found in the 34 adults who had resided permanently in the area.

No antibodies to RVF were found in children, in contrast to the 5·8% positive children of the northern areas recorded by SMITHBURN *et al.* (1959). These authors did not present results for children south of Mtubatuba and reported the absence of RVF antibodies from 11 adults from Icubu Lake, immediately south of Richards Bay. The results of our study, however, indicated that 5·8% of the 34 adults who had resided permanently in the area had antibodies to this virus.

The results of both studies show that Wesselsbron viral activity is lower to the south than to the north of Mtubatuba. We report that 5·2% of the children and 8·8% of the group of permanent adult residents were positive compared to the 1·3% for children and the 13·2% for adults found by SMITHBURN *et al.* (1959). It is possible that the habitats at Richards Bay are suitable for *Aedes* vectors although no *Ae. circumluteolus*, which is the main vector in northern Natal (SMITHBURN *et al.*, 1957; MCINTOSH, 1980), were caught here.

The overall situation for Banzi virus recorded in the present study (Table 3) does not compare well with that reported by SMITHBURN *et al.* (1959). They found that 29·4% adults and 15·4% children living at Icubu lake were positive, while those living inland at Eshowe (28°56′S; 31°28′E) were negative. We found that only 5·8% of the permanent adult residents and 2·1% of the children at Richards Bay were positive, which is significantly lower than the rate for Icubu Lake residents.

The vector of Banzi virus is *C. rubinotus* (JUPP *et al.*, 1976) and wherever the habitat is suitable for this mosquito the local human population can be expected to show a high positivity rate for antibodies to this virus. The results for West Nile virus indicate that it is not active, if present, south of Mtubatuba.

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