

Seroepidemiological Studies in Oriental Mindoro (Philippines) – Prevalence of Mosquito-borne Parasitoses

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Introduction

According to the literature of the past twenty years, at least six human pathogens transmitted by mosquitos (*Culicidae*) occur in Mindoro island (area: 10,245 km², 803,243 inhabitants) situated 130 km south of Manila (Philippines) (Fig. 1): Among them there are two viruses causing Dengue (hemorrhagic) fever (10, 12) and Japanese (B) encephalitis (4), respectively, three protozoan species i. e. *Plasmodium falciparum* (malignant tertian malaria), *P. vivax* (benign tertian malaria), *P. malariae* (quartan malaria) (5, 13, 16) and the nematode *Wuchereria bancrofti*, the causing organism of lymphatic filariasis/elephantiasis (7, 8, 9, 11, 15).

During the last decades epidemiological studies were carried out nearly exclusively in the western regions of Mindoro (Occidental Mindoro). In contrast, we examined serum specimens from patients resident in Oriental Mindoro (area: 4,364.7 km², 536,000 inhabitants, capital city: Calapan).

It was already in 1993 when RADDA et al. (10) published the first results of this epidemiological study on the occurrence of Dengue (hemorrhagic) fever and Japanese encephalitis. This serological survey revealed a seroprevalence rate of 36% and 29% regarding Dengue virus and Japanese B encephalitis virus antigen, respectively.

The following paper summarizes the results of serological examinations concerning mosquito-borne parasitoses (malaria, lymphatic filariasis) on one hand and presents on the other hand a complete list of *Culicidae* species (including those acting as vectors) occurring in Mindoro.

An additional paper will report on the seroepidemiological survey for toxoplasmosis, bilharziosis, fasciolosis, echinococcosis, cysticercosis and toxocarosis.

Materials and methods

Sera from 163 out-patients, the majority suffering from fever, from the Provincial Hospital in Calapan/Oriental Mindoro were collected between November 1991 and October 1992. Sera were stored at -20° C until serological examination which was carried out in Vienna/Austria. Personal (age, sex) and epidemiological (origin) data were collected from most patients (Figures 1, 2).

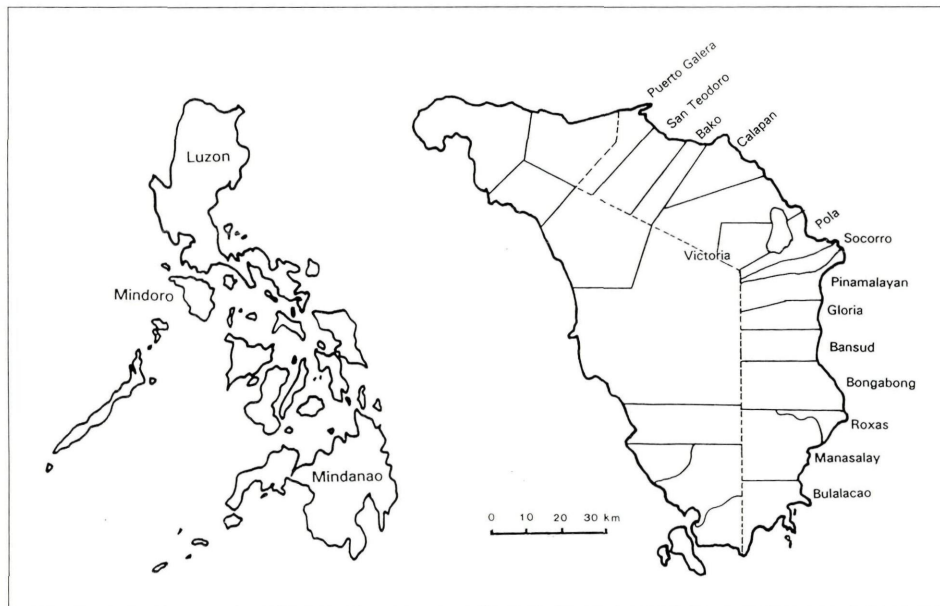


Figure 1:
Map of Mindoro.

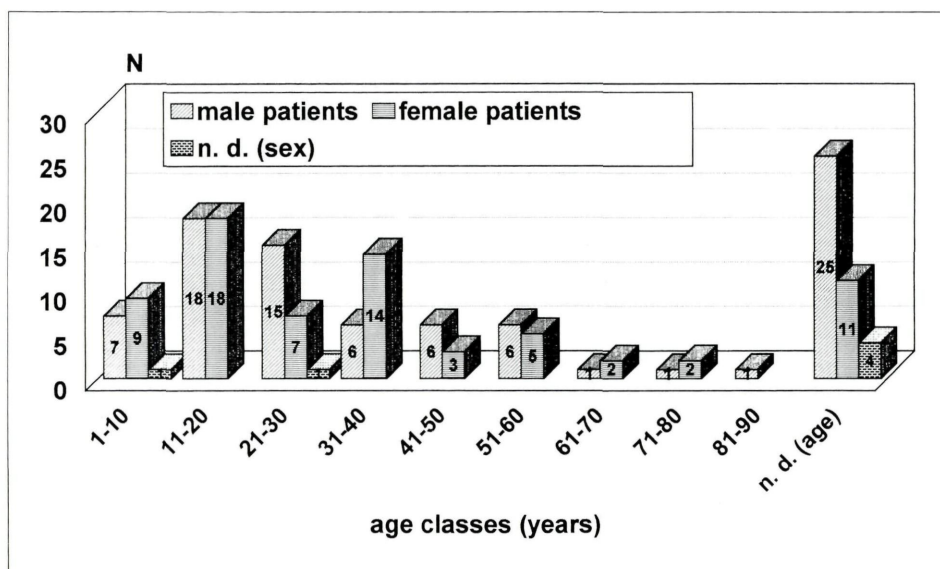


Figure 2:
Age and sex distribution of patients attending the Provincial Hospital in Calapan.
n. d.: no data available.

Serological tests

Indirect immunofluorescence test (IIFT) for the detection of antibodies against *Plasmodium falciparum* antigen

Antigen:

Falciparum-Spot IF (bioMérieux).

Sera:

Positive, negative control and test sera were diluted (1:16, 1:64, 1:128) in phosphate buffered saline (PBS, pH 7.2).

Conjugate:

FITC-conjugated antihuman IgM + IgA + IgG (Behring/Germany), dilution: 1:30, buffer: PBS.

Enzyme-linked immunosorbent (ELISA) for the detection of antibodies against *Dipetalonema viteae* antigen

Antigen:

Water-soluble extract from adult male and female *Dipetalonema viteae* harvested from experimentally infected gerbils (*Meriones unguiculatus*) kindly provided by Prof. Dr. R. Lucius/Institute of Parasitology, University of Hohenheim/Stuttgart (Germany). Protein content: 3.6 µg/ml.

Sera:

Positive, negative control and test sera were diluted 1:100 in ELISA-buffer (PBS [pH 7.2] + 5% milk powder + 0.05% Tween 20).

Conjugate:

Peroxidase-conjugated antihuman-IgG (Jackson ImmunoResearch/USA), dilution: 1:6000 (ELISA-buffer).

Substrate:

H₂O₂ + 5 amino-salicylic acid.

Interpretation of test results:

Enzymatic reactions were read at 450 nm photometrically, the analysis of test results was carried out according to the formula of ZAHNER et al. (18). Indices were expressed as antibody units (AU) based on a (laboratory internal) positive standard serum with 100 AU. Sera with an index lower than 50 AU were considered as negative, sera with an index

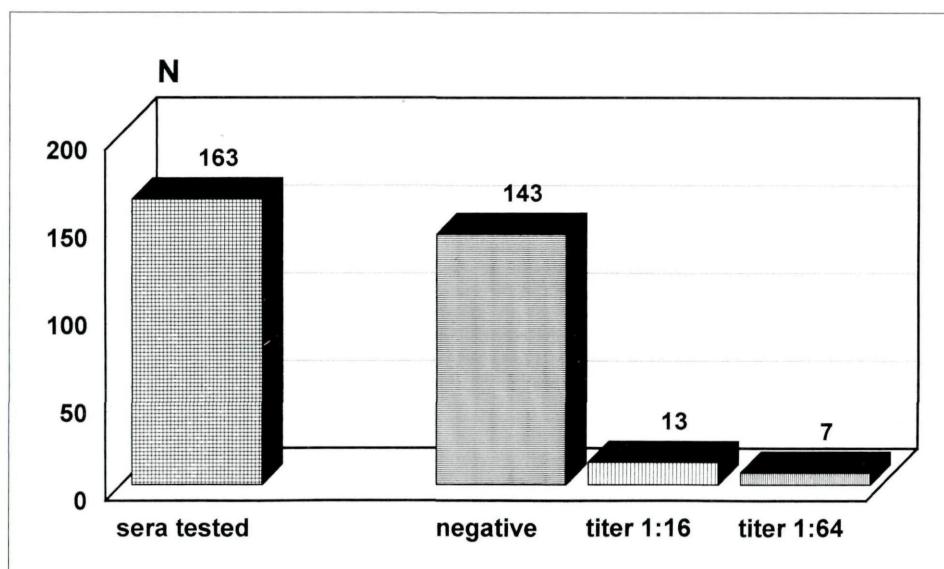


Figure 3:
Test results (titer) of *Plasmodium falciparum* IIFT.

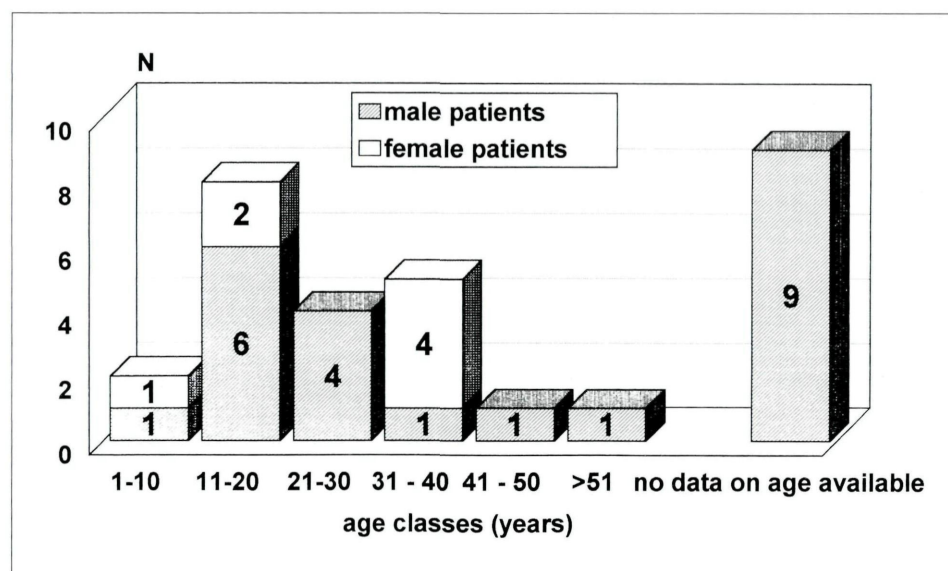


Figure 4:
Test results of
Dipetalonema viteae ELISA.

males, 7 from female patients. The average age of patients was 25.4 (4 – 84) years. Eight patients derived from the district Naujan, six from the districts Calapan and Socorro, respectively, three from the district Pola, two from the districts Abra (Puerto Galera) and Baco, respectively, one patient had his origin in the district Pinamalayan, from two patients no data on their origin were available.

between 51 and 70 AU and between 71 and 90 AU were considered as low positive and positive, respectively, and sera with indices higher than 90 AU were considered highly positive.

List of *Culicidae* occurring in Mindoro

The list of *Culicidae* species is based on the following papers:

BALTAZAR (3), CABRERA and ARAMBULO (5), ISHII et al. (9), SCHULTZ (12), SMRKOVSKI et al. (13), SUGURI et al. (15), WOOSTER and RIVERA (17) (Tab. 1).

Results

Malaria

163 sera of out-patients from the Provincial Hospital in Calapan were tested for specific antibodies against *Plasmodium falciparum* antigen by IIFT, 143 (87.7%) sera were negative, 13 (8.0%) sera were borderline positive (titer 1:16), 7 (4.3%) sera showed a titer of 1:64 (Fig. 3). Out of the latter group four patients were males (17, 20, 55 and 84 years old), three were females (5, 31, 59 years old) (Tab. 2). Three patients had their origin in the district Socorro, three patients derived from the districts Abra (Puerto Galera), San Teodoro and Pinamalayan, respectively, in one case no data on the origin were available (Fig. 1).

Lymphatic Filariasis

The results of *Dipetalonema viteae* ELISA can be seen in Figures 4, 5 and 6. 112 (68.7%) out of 163 tested sera were negative, 21 sera (12.9%) were low positive (61 – 70 AU), 30 sera proved to be positive (n = 9 = 5.5%) or highly positive (n = 21 = 12.9%). 23 out of 30 positive (and highly positive) sera were from

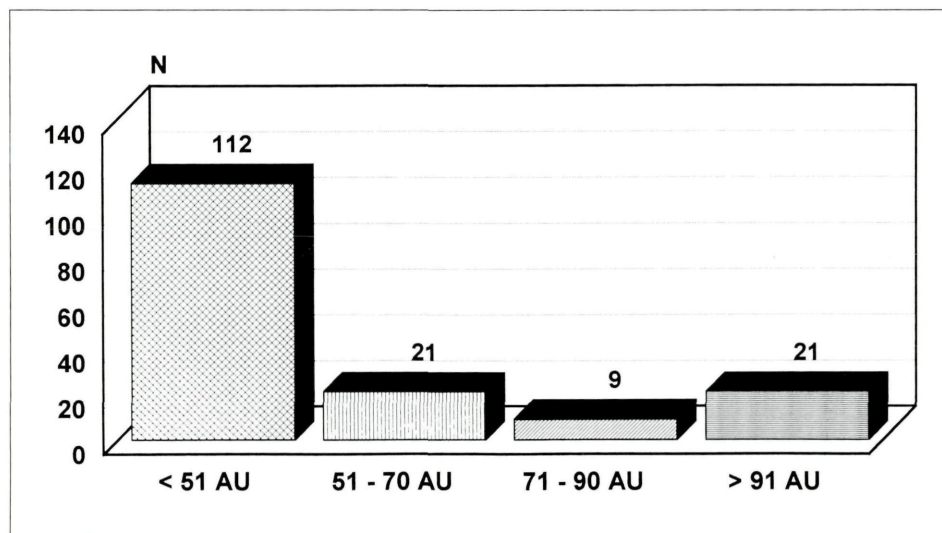


Figure 5:
Age and sex distribution of patients with antibodies against *Dipetalonema viteae* antigen.

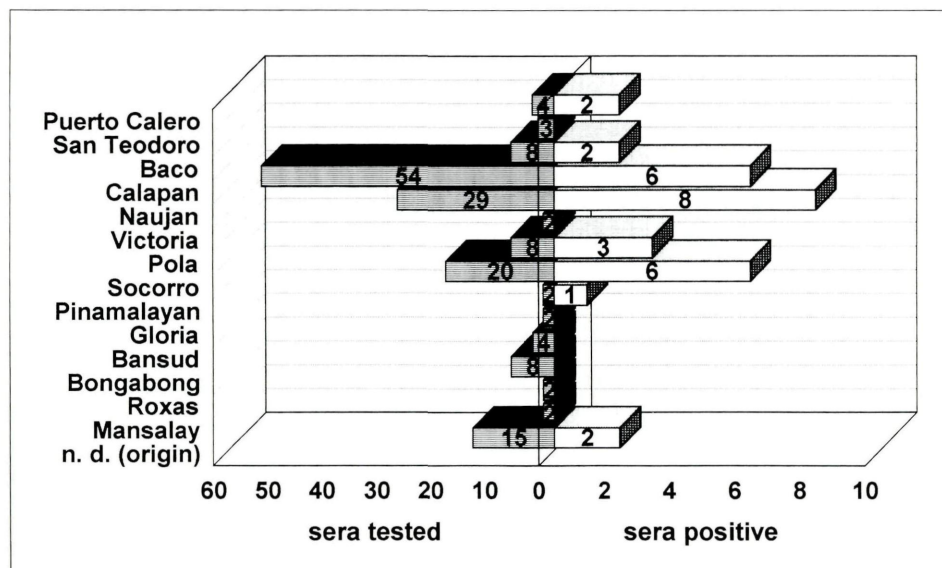


Figure 6:
Origin of patients with antibodies against *Dipetalonema viteae* antigen (white blocks).

P. ovale is not prevalent in Mindoro, but does occur on the island Palawan (CABRERA and ARAMBULO, 1977).

Based on the premise that an IIFT titer of 1 : 64 or higher is a clear indication of a latent or an evolutive *P. falciparum* malaria (1, 2) our epidemiological study revealed a malaria seroprevalence of 4.3%. This value is only slightly higher than that obtained by SMRKOVSKI et al. (13, 14) and, thus, in fact comparable to SMRKOVSKI's findings. The validity of this value may be due to two reasons:

Discussion

The aim of the study was an assessment of the recent epidemiological situation of mosquito-borne parasitoses i. e. of malaria and lymphatic filariasis, in Oriental Mindoro on the basis of a seroepidemiological survey, results of a seroepidemiological study for Dengue (hemorrhagic) fever and Japanese encephalitis in Oriental Mindoro had been recently published (10). Additionally, we have tried to give an overall view of the *Culicidae* fauna prevalent in Mindoro.

Malaria

Until about 50 years ago, Mindoro was considered a high risk area for malaria, and this resulted in a delayed social and economic development. Only after the first and particularly after the second World War an increase of the number of people moving from other parts of the archipelago (mainly from the Central Talaglog region of Luzon) to Mindoro occurred. Intensive efforts of health authorities to control malaria were successful and had led to an essential reduction of the incidence of the disease. However, Mindoro was still considered as a "hard-core malarious area" with "on-going persistent transmission" (6) until 1972.

At the beginning of the 1980s SMRKOVSKI et al. (13) carried out an epidemiological survey in the northern parts of Occidental-Mindoro examining blood films of 600 persons: 2.8% of patients proved to be infected with *Plasmodium falciparum*, 4.3% with *P. vivax* and 0.7% with *P. malariae*. SY et al. (16), however, ascertained a seroprevalence rate of 80% in the southern parts of Occidental-Mindoro.

1. The high specificity of the IIFT used which generally does not detect infections with *P. vivax* or *P. malariae*.

2. The very long persistence of specific antibodies in serum samples, thus, epidemiological studies based on serological tests tend to reveal higher prevalence rates than that obtained on direct examination of blood films.

The main vector for the transmission of malaria in Mindoro is *Anopheles (Cellia) flavirostris* (*A. minimus flavirostris*), a species particularly breeding in clear brooks of the hilly countryside, whereas *A. (C.) maculatus* and *A. (C.) mangyanus* represent the main vectors in higher altitudes (17), in coastal areas *A. litoralis* proved to be the important vector for malaria transmission (Tab. 1). *A. (C.) flavirostris* has its peak of activity during the night and thus it does not represent a big problem for the tourists concerning effective prophylaxis by use of mosquito-nets impregnated with repellents. The risk of infection remains limited to the indigenous Mangyan population and the socially weaker levels of lowland dwellers ("Filipinos").

Lymphatic filariasis

First investigations on the prevalence and incidence of *Wuchereria bancrofti* – the only filaria species occurring on Mindoro – have been carried out during the 1970s (5). At the beginning of the 1980s it was particularly ENARSON and ENARSON (7) who studied the indigenous population (Mangyan) for *Wuchereria bancrofti* infections: In total 972 persons were examined for microfilariæmia by direct examination methods (blood films), 136 persons (14%) proved to be carriers of microfilariæ. Two foci were described by the authors (7): the valley of the Alag River (district San Teodoro) and the southeastern districts Bongabon, Mansalay and Bulalacao.

In our study 163 patients of the Provincial Hospital in Calapan were tested for antibodies against filarial (i. e. *Dipetalonema viteae*) antigen, 112 sera were serologically negative, 21 (12.9%) sera showed borderline-positive reactions (51 – 69 AU), 30 sera (18.4%) were positive (> 70 AKE). The calculated seroprevalence rate of 18.4% proved to be similar to that obtained by ENARSON and ENARSON (7) by direct examination methods. This was a rather surprising result since we had expected a much higher seroprevalence rate due to the use of the heterologous and thus cross-reacting antigen (deriving from a species which is apathogenic to man). On the other hand *Wuchereria bancrofti* is the only species of filariæ occurring in the human population in Mindoro, so that we can assume that our positive ELISA results are really due to this parasite.

Male patients (76.7%) proved to be infected four times higher than females (23.3%), an observation which was also reported by other authors (7, 8). The average age of serologically positive patients was 24.5 years, the youngest was 4, the oldest 84 years. The 30 *Dipetalonema viteae*-positive patients derived from the districts situated in the northeast of Mindoro (Abra [Puerto Galera], Baco, Calapan, Naujan, Pola, Socorro and Pinamalayan).

Patients with clinical manifestations of lymphatic filariasis could not be detected in our group of patients.

Out of 101 species of *Culicidae* so far found in Mindoro (Tab. 1) only *Aedes (Finlaya) poecilus*, *Culex quinquefasciatus* and *Anopheles (Cellia) flavirostris* have proved to be vectors of *Wuchereria bancrofti* filariasis. The main vector is *Aedes (Finlaya) poecilus*, an enormously anthropophilic mosquito, particularly breeding in abaku and banana plants (11). Due to the fact that *A. poecilus* has its peak at midnight the risk of infection is very low to tourists since they are capable of carrying out effective prophylaxis with impregnated mosquito nets.

Table 1:
Culicidae occurring in Mindoro

Spezies	Vector of
SUBFAMILY ANOPHELINAE	
Anopheles (Anopheles) fragilis	(THEOBALD, 1903)
Anopheles (Anopheles) umbrosus	(THEOBALD, 1903)
Anopheles (Cellia) annularis	VAN DER WULP, 1894
Anopheles (Cellia) filipinae	MANALANG, 1930
Anopheles (Cellia) flavirostris	(LUDLOW, 1914)
Anopheles (Cellia) litoralis	KING, 1932
Anopheles (Cellia) ludlowae	(THEOBALD, 1903)
Anopheles (Cellia) maculatus	THEOBALD, 1903
Anopheles (Cellia) mangyanus	(BANKS, 1906)
Anopheles (Cellia) philippinensis	LUDLOW, 1902
Anopheles (Cellia) subpictus	GRASSI, 1899
Anopheles (Cellia) tessellatus	(THEOBALD, 1901)
Anopheles (Cellia) vagus	DENITZ, 1902
	Japanese B Encephalitis
SUBFAMILY CULICINAE	
Aedeomyiini	
Aedomyia (Aedomyia) catacticta	KNAB, 1909
Aedeini	
Aedes (Aedimorphus) alboscuteallatus	(THEOBALD, 1905)
Aedes (Aedimorphus) lowisi	(THEOBALD, 1910)
Aedes (Belkinius) aurotaeniatus	EDWARDS, 1922
Aedes (Edwardsaedes) imprimens	(WALKER, 1860)
Aedes (Finlaya) poicilius	(THEOBALD, 1903)
Aedes (Geoskusea) baisasi	KNIGHT & HULL, 1951
Aedes (Lorrainea) amesi	(LUDLOW, 1903)
Aedes (Mucidus) laniger	(WIEDEMANN, 1820)
Aedes (Ochlerotatus) vigilax ludlowae	(BLANCHARD, 1905)
Aedes (Paraedes) ostentatio	(LEICESTER, 1908)
Aedes (Paraedes) pagei	(LUDLOW, 1911)
Aedes (Scutomyia) bambusicola	KNIGHT & ROZEBOOM, 1946
Aedes (Scutomyia) impatibilis	(WALKER, 1859)
Aedes (Scutomyia) paullusi	STONE & FARNER, 1945
Aedes (Stegomyia) albopictus	(SCUSE, 1895)
Aedes (Stegomyia) alcasidi	HUANG, 1972
Aedes (Stegomyia) gardneri	(LUDLOW, 1905)
Aedes (Verralina) campylosylus	LAFFOON, 1946
Aedes (Verralina) dux	DYAR & SHANNON, 1925
Aedes (Verralina) hamistylus	LAFFOON, 1946
Aedes (Verralina) macrodixoa	DYAR & SHANNON, 1925
Aedes (Verralina) margarsen	DYAR & SHANNON, 1925
Armigeres (Armigeres) baisasi	STONE & THURMAN, 1958
Armigeres (Armigeres) joloensis	(LUDLOW, 1904)
Armigeres (Armigeres) manalangii	BAISAS, 1935
Armigeres (Leicesteria) digitatus	(EDWARDS, 1914)
Armigeres (Leicesteria) magnus	(THEOBALD, 1908)
Armigeres (Leicesteria) omissus	(EDWARDS, 1914)
Heizmannia (Heizmannia) scintilla	LUDLOW, 1905
Culicini	
Culex (Culex) annulirostris	SKUSE, 1889
Culex (Culex) bitaeniorhynchus	GILES, 1901
Culex (Culex) fuscocephalea	THEOBALD, 1906
Culex (Culex) gelidus	THEOBALD, 1901
Culex (Culex) incognitus	BAISAS, 1938
Culex (Culex) infula	THEOBALD, 1901
	Japanese B Encephalitis

Summary

In order to obtain an overview on the recent situation of mosquito-borne parasitic infections, malaria and bancroftian filariasis, in Oriental Mindoro (Philippines) a sero-epidemiological study was carried out with sera obtained from 163 patients attending the Provincial Hospital in Calapan. Sera were examined on the prevalence of specific antibodies against *Plasmodium* and filarial antigens using an indirect immunofluorescence test (IIFT) with *Plasmodium falciparum*-antigen on one hand and an enzyme-linked immunosorbent assay (ELISA) with *Dipetalonema viteae*-antigen on the other. Seven (4.3%) out of 163 sera were positive in the *P. falciparum*-IIFT, 30 sera (18.4%) showed positive reactions in *Dipetalonema viteae*-ELISA. A list comprising all species of *Culicidae* occurring in Mindoro (including those species acting as vectors of infectious diseases) is presented.

Key words

Oriental Mindoro, seroepidemiological study, malaria, filariasis.

Zusammenfassung

Seroprävalenzuntersuchungen in Ost-Mindoro/Philippinen: Vorkommen und Häufigkeit von durch Stechmücken (Culicidae, Diptera) übertragenen Parasitosen

Ziel der vorliegenden Arbeit war die Erhebung der rezenten epidemiologischen Situation der durch Stechmücken (*Culicidae*) übertragenen Parasitosen — Malaria und lymphatische Filariose — in Ost-Mindoro/Philippinen auf der Basis von Seroprävalenzuntersuchungen.

Culex (Culex) pseudovishnui	COLLESS, 1957	
Culex (Culex) quinquefasciatus	SAY, 1823	Filariosis
Culex (Culex) sitiens	WIEDEMANN, 1828	
Culex (Culex) tritaeniorhynchus	GILES, 1901	Japanese B Encephalitis
Culex (Culex) vishnui	THEOBALD, 1901	Japanese B Encephalitis
Culex (Culex) whitmorei	(GILES, 1904)	
Culex (Culiciomyia) fragilis	LUDLOW, 1903	
Culex (Culiciomyia) nigropunctatus	EDWARDS, 1926	
Culex (Culiciomyia) pallidithorax	THEOBALD, 1905	
Culex (Culiciomyia) scanloni	BRAM, 1967	
Culex (Culiciomyia) spathifurca	(EDWARDS, 1915)	
Culex (Eumelanomyia) brevipalpis	(GILES, 1902)	
Culex (Eumelanomyia) foliatus	BRUG, 1932	
Culex (Eumelanomyia) hinglungensis	CHU, 1957	
Culex (Eumelanomyia) latifolius	DELFINADO, 1966	
Culex (Eumelanomyia) laureli	BAISAS, 1935	
Culex (Lophoceraomyia) gibbulus	DELFINADO, 1966	
Culex (Lophoceraomyia) infantulus	EDWARDS, 1922	
Culex (Lophoceraomyia) josephinae	BAISAS, 1935	
Culex (Lophoceraomyia) kuhnsi	KING & HOOGSTRAAL, 1955	
Culex (Lophoceraomyia) lavatae	STONE & BOHART, 1944	
Culex (Lophoceraomyia) macdonaldi	COLLESS, 1955	
Culex (Lophoceraomyia) mammilifer	(LEICESTER, 1908)	
Culex (Lophoceraomyia) minor	(LEICESTER, 1908)	
Culex (Lophoceraomyia) rubithoracis	(LEICESTER, 1908)	
Culex (Lophoceraomyia) uniformis	(THEOBALD, 1905)	
Culex (Lophoceraomyia) halifaxi	THEOBALD, 1903	

Ficalbiini

Mimomyia (Etorleptomyia) luzonensis	(LUDLOW, 1905)
Mimomyia (Mimomyia) chamberlaini	LUDLOW, 1904
Mimomyia (Ingramia) deguzmanae	(MATTINGLY, 1957)

Hodgesinii

Hodgesia malayi	LEICESTER, 1908
Hodgesia quasianguinae	LEICESTER, 1908

Mansoniini

Coquillettima crassiceps	VAN DER WULP, 1881
Mansonia (Mansonioides) bonnae	EDWARDS, 1930
Mansonia (Mansonioides) dives	(SCHINER, 1868)
Mansonia (Mansonioides) uniformis	(THEOBALD, 1901)

Orthopodomyiini

Orthopodomyia anopheloides	(GILES, 1903)
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Sabethini

Tripteroides (Tripteroides) powelli escodae	BAISA & PAGAYON, 1953
Tripteroides (Tripteroides) powelli laffooni	BAISA & PAGAYON, 1953
Uranotaenia (Pseudoficalbia) abstrusa	PEYTON, 1977
Uranotaenia (Pseudoficalbia) confusa	PEYTON, 1977
Uranotaenia (Pseudoficalbia) harrisoni	PEYTON, 1977
Uranotaenia (Pseudoficalbia) modesta	LEICESTER, 1908
Uranotaenia (Pseudoficalbia) obscura	EDWARDS, 1915
Uranotaenia (Pseudoficalbia) rossi	DELFINADO, 1966
Urotaenia (Urotaenia) annandalei	BARRAUD, 1926
Urotaenia (Urotaenia) arguellesi	(BAISAS, 1935)
Urotaenia (Urotaenia) clara	DAYAR & SHANNON, 1925
Urotaenia (Urotaenia) lateralis	LUDLOW, 1905
Urotaenia (Urotaenia) mendiola	BAISAS, 1935
Urotaenia (Urotaenia) nivea	LEICESTER, 1908

SUBFAMILY TOXORHYNCHITINAE

Toxorhynchites (Toxorhynchites) amboinensis	(DOLESCHALL, 1857)
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In den Jahren 1991 und 1992 wurden insgesamt 163 Seren von ambulanten Patienten des Provinzspitals von Calapan auf das Vorhandensein von *Plasmodium*- (mittels eines Indirekten Immunfluoreszenztests [IIFT] unter Verwendung von *Plasmodium falciparum*-Antigen) und Filarien-Antikörper (mittels eines Enzymimmuntests [ELISA] unter Verwendung von *Dipetalonema viteae*-Antigen) getestet. Sieben Seren (4,3%) waren im *P. falciparum*-IIFT und 30 Seren (18,4%) im *Dipetalonema viteae*-ELISA positiv. Die vorliegende Arbeit wird abgerundet durch eine vollständige Liste der Culicidenfauna Mindoros (inklusive der als Vektoren von Infektionskrankheiten fungierenden Stechmückenarten).

Schlüsselwörter

Ost-Mindoro, seroepidemiologische Studie, Malaria, Filariose.

Acknowledgement

We acknowledge the excellent technical assistance of Mrs. J. Will and Mrs. M. Gerami.

Table 2:

Data on the origin, age, sex and clinical symptoms of patients with positive malaria serology.

Nr.	Pat.	Sex	Age	Village	District	Titer	Symptoms
1	C. B.	M	20	Baladero	Puerto Galera	1 : 64	no data
2	V. C.	W	31	no data	San Teodoro	1 : 64	no data
3	J. A.	M	17	Pasi	Socorro	1 : 64	fever
4	M. T.	W	5	Calubayan	Socorro	1 : 64	fever
5	L. G.	W	59	Happy Valley	Socorro	1 : 64	fever
6	C. H.	M	84	no data	Pinamalayan	1 : 64	fever
7	R. M.	M	55	no data	no data	1 : 64	no data

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Zeitschrift/Journal: [Mitteilungen der Österreichischen Gesellschaft für Tropenmedizin und Parasitologie](#)

Jahr/Year: 1995

Band/Volume: [17](#)

Autor(en)/Author(s): Radda Alfred C., Auer Herbert, Escalona T.G., Hartnett I., Aspöck Horst

Artikel/Article: [Seroepidemiological Studies in Oriental Mindoro \(Philippines\) - Prevalence of Mosquito-borne Parasitoses. 159-168](#)