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Seroepidemiological Studies in Oriental Mindoro (Philippines) — Prevalence of Parasitic Zoonoses

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Introduction

Mindoro is one of the largest islands (area: 10,245 km^2; 803,243 inhabitants) of the Philippine archipelago and is situated about 130 km far from Manila. In contrast to the western part (Occidental Mindoro) of the 150 km long and 45 km broad island, only few data on the prevalence of parasitic diseases (i. e. malaria [9], filariosis [2, 4], bilharziosis [4]) are available from Oriental Mindoro.

In order to get an overview on the recent epidemiological situation of infectious diseases in general and on parasitoses in particular in Oriental Mindoro we collected sera from patients of the Provincial Hospital in Calapan, the capital city of Oriental Mindoro, between 1991 and 1992. These serum specimens were subsequently examined for the presence of specific antibodies against two viral and several parasitic antigens. The results of our study concerning mosquito-borne viral (Dengue fever, Japanese Encephalitis) and parasitic (malaria, lymphatic filariosis) diseases have already been published (6) or are in press (7).

The present paper summarizes the few published epidemiological data on one hand and reports the first results of a serological survey for toxoplasmosis, bilharziosis, fasciolosis, echinococcosis, cysticercosis, trichinosis and toxocarosis in Oriental Mindoro on the other hand.

Materials and methods

Patients

Sera from patients most of them suffering from fever and attending the Provincial Hospital in Calapan were collected between November 1991 and October 1992 and stored at -20° C until serological examination which was carried out in Vienna/Austria (Tab. 1).

Serological tests (Tab. 1)

1. Indirect Immunofluorescence test (IIFT) for the detection of antibodies against *Toxoplasma gondii* antigen:

Antigen:
Trophozoites of *Toxoplasma gondii* from tissue culture (Hep-2 cell).

Sera:
Positive, negative control and test sera were diluted (1 : 16, 1 : 64, 1 : 256, 1 : 1024, 1 : 4096) in phosphate buffered saline (PBS, pH 7.2).

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

2. Enzyme-linked immunosorbent (ELISA) for the detection of antibodies against *Schistosoma mansoni* (arc 4) antigen:

Antigen:
Antigene bilharzien (Institute Pasteur), protein content: 12.5 µg/ml, buffer: bicarbonate/carbonate pH 9,6.

Sera:
Positive, negative control and test sera were diluted 1 : 200 in ELISA-buffer (PBS [pH 7.2] + 5% milk powder + 0.05% Tween 20).

Conjugate:
Peroxidase-conjugated antihuman-IgG (Jackson ImmunoResearch/USA), dilution: 1 : 3000 (ELISA-buffer).

Substrate:
H_2O_2 + 5 amino-salicylic acid.

3. Enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies against *Fasciola hepatica* (arc 2) antigen:

Antigen:
Antigene distomien (Institute Pasteur), protein content: 50 µg/ml, buffer: see 2.

Sera:
Positive, negative control and test sera were diluted 1 : 400 in ELISA-buffer.

Conjugate and Substrate: see 2.

4. Indirect haemagglutination test (IHA) for the detection of antibodies against *Echinococcus granulosus:*

Echinococcose-HA-Kit (BioMérieux/France).

5. Enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies against *Taenia solium* antigen:

Cysticercosis *(Taenia solium)* Serology (LMD Laboratories/USA).

6. Westernblot (WB) for the detection of antibodies against *Taenia solium* (26 + 8 kd) antigen:

The test was carried out according to GOTTSTEIN et al. (1987) (3).

7. Enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies against *Toxocara canis* E/S antigen:

Antigen:
Bordier affinity products, protein content: 100 µg/ml, buffer: see 2.

Sera:
Positive, negative control and test sera were diluted 1 : 200 in ELISA-buffer.

Conjugate and substrate: see 2.

8. Enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies against *Trichinella spiralis* E/S antigen:

Trichinella Serology (LMD Laboratories/USA).

9. Indirect immunofluorescence test (IIFT) for the detection of antibodies against *Trichinella spiralis* antigen:

Antigen:
Cryosections of *Trichinella spiralis* larvae on glass slides.

Sera and conjugate: see 1.

Table 1:

Number of sera tested, serological tests carried out and test results.
IIFT: indirect immunofluorescence test · ELISA: enzyme-linked immunosorbent assay

Antigen	Test	Test positive	Sera tested	Sera positive
Toxoplasma gondii (trophozoites of tissue culture)	IIFT (own preparation)	Titer: 1 : 16 or higher	239	76 (32%)
Schistosoma mansoni (antigene biharzien – Institute Pasteur)	ELISA (own preparation)	> 30 AU*	162	0
Fasciola hepatica (antigene distomien – Institute Pasteur)	ELISA (own preparation)	> 30 AU*	162	0
Echinococcus granulosus	IHA (BioMérieux)	Titer 1 : 100 or higher	162	0
Taenia solium	ELISA (LMD Laboratories)	> 0.5 OD**	162	4
Taenia solium (26, 8 kd-bands)	WB (according to GOTTSTEIN et al. [3])	specific bands detectable	4	0
Toxocara canis (E/S) (Bordier affinity products)	ELISA (own preparation)	> 60 AU	162	108 (67%)
Trichinella spiralis (E/S)	ELISA (LMD Laboratories)	> 0.5 OD**	162	5
Trichinella spiralis (larvae)	IIFT (own preparation)	Titer 1 : 16 or higher	5	0

* AU: antibody units based on a (laboratory internal) positive standard with 100 AU.
** OD: optional density.

Results and discussion

As far as we know this study is the first epidemiological one concerning the prevalence of *Toxoplasma gondii* in man in Mindoro in general and in Oriental Mindoro in particular.

Toxoplasmosis

The results of this study (Fig. 1, Tab. 1) show that only 76 (= 32%) out of 239 sera exhibited specific antibodies against *Toxoplasma gondii* antigen. Due to the fact that the climatic conditions (temperature: 20 – 30° C, atmospheric humidity: 65 – 90%) in Oriental Mindoro favour the survival of *Toxoplasma* oocysts in the environment, the infection rate of 32% seems rather low and, thus, it is nearly comparable to prevalence rates obtained in some "developed" countries (1). Essential differences of the infection rates between males and females or between the age groups, respectively, could not be observed (Fig. 1). It is of interest that the infection rates in higher age groups are not significantly higher than in younger people.

Bilharziosis

For more than 50 years the endemic occurrence of *Schistosoma japonicum* (dogs and pigs as reservoir hosts) in Oriental Mindoro (Lake Naujan) has been known (4). After World War II the prevalence rates in man were 10.4% in the case of the district Naujan. The Schistosomiasis Control Council estimates that 7.94% of the 118,149 inhabitants of the Naujan, Victoria, Socorro and Pola municipalities (= 30.4% of the total population of Oriental Mindoro) are de facto infected with *S. japonicum* (4).

In spite of the fact that also patients deriving from the *Schistosoma japonicum* endemic areas were included in our study none of the 162 serum specimens tested by *Schistosoma* ELISA showed a positive reaction (Tab. 1). The only explanation for this finding is the high degree of specificity of the used antigen (arc 4 antigen) which was prepared from *S. mansoni*; homologous *S. japonicum* antigen was not available for this survey.

Fasciolosis

No more than five cases of human fasciolosis have so far been documented in the Philippines until 1985 (4), so that it has to be considered a very rare disease in the Philippines, and thus also in Mindoro. Our study confirmed this finding, since none of the 162 sera tested contained specific antibodies against *Fasciola hepatica* antigen although we used a highly sensitive *Fasciola hepatica* (arc 2) ELISA (Tab. 1).

Echinococcosis

The epidemiological situation of cystic echinococcosis in man in the Philippines is characterized by an extreme scarcity, only three autochthonous cases have been documented (4). Based on our serological examination we also have no indication for the endemic occurrence of cystic echinococcosis in Oriental Mindoro (Tab. 1).

Cysticercosis

According to the literature there is no real indication for the occurrence of *Taenia solium* (and *Taenia solium* cysticercosis of man) in Oriental Mindoro until today (4). Our serological survey confirmed this observation. Although four out of 162 tested sera proved to be positive in the *Taenia solium* ELISA, these positive reactions could not be confirmed in the *Taenia solium* Westernblot (Tab. 1).

Trichinosis

As far as we know there is no evidence of the autochthonous occurrence of *Trichinella spiralis* s. l. in the Philippines (4). Our serological survey also revealed no indication for human infections with this parasite. Although 5 out of 162 sera showed positive reactions in *Trichinella spiralis* ELISA using E/S antigens, these results could not be confirmed by indirect immunofluorescence test (Tab. 1).

Toxocarosis

Toxocara canis is a very common parasite of dogs in the Philippines, infection rates of 4 to 13.6% are known, a high degree of environmental contamination by *Toxocara* eggs must be assumed (4). Data on the infection rate of man, however, have not been available from the Philippines until today. In our study 108 out of 162 sera showed positive reactions revealing a seroprevalence of 67% in Oriental Mindoro (Tab. 1) which is comparable to the prevalence rates obtained in other countries of low hygienic standard in the tropics and subtropics (5, 8). Essential differences concerning the infection rate could not be observed either between males and females or between the different age groups, respectively (Fig. 2).

Summary

A serological survey for parasitic zoonoses was carried out in Oriental Mindoro/Philippines. Sera collected from patients attending the Provincial Hospital in Calapan were examined for the presence of specific antibodies against *Toxoplasma gondii, Schistosoma mansoni, Fasciola hepatica, Echinococcus granulosus, Taenia solium, Trichinella spiralis* and *Toxocara canis* antigen. 76 out of 239 sera (= 32%) and 108 out of 162 (= 67%) were positive in a *T. gondii* IIFT or in a *T. canis* ELISA, respectively. Specific antibodies against other parasitic antigens could not be detected in the serum specimens.

Key words

Oriental Mindoro, seroepidemiological study, toxoplasmosis, bilharziosis, fasciolosis, echinococcosis, cysticercosis, toxocarosis, trichinellosis.

Zusammenfassung

Seroprävalenzuntersuchungen in Ostmindoro/Philippinen: Vorkommen und Häufigkeit parasitärer Zoonosen

Im Rahmen einer seroepidemiologischen Studie wurden Blutproben von Patienten, die das Provinzspital von Calapan, der Hauptstadt Ostmindoros/Philippinen, aufgesucht hatten, auf spezifische Antikörper gegen folgende Antigene getestet: *Toxoplasma gondii, Schistosoma mansoni, Fasciola hepatica, Echinococcus granulosus, Taenia solium, Trichinella spiralis, Toxocara canis*. 76 von insgesamt 239 Seren (= 32%) und 108 von 162 (= 67%) erwiesen sich

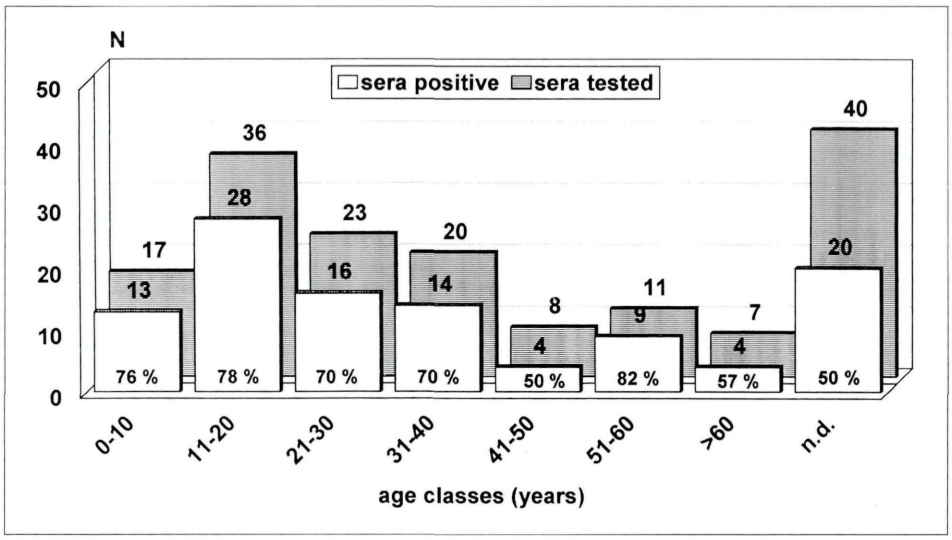

Figure 1:

Age distribution of patients (N = 239) examined in the indirect immunofluorescence test (IIFT) for the presence of specific antibodies against *Toxoplasma gondii* antigen.
n. d.: no data on the age of patients available.

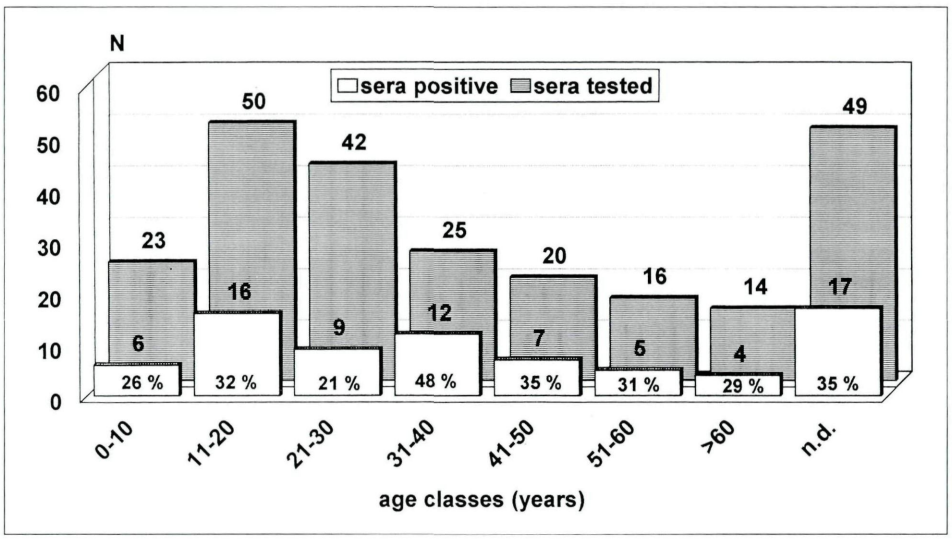

Figure 2:

Age distribution of patients (N = 162) examined in the enzyme-linked immunosorbent assay (ELISA) for the presence of specific antibodies against *Toxocara canis* E/S antigen.
n. d.: no data on the age of patients available.

im IIFT mit *T. gondii*- bzw. im ELISA mit *T. canis*-Antigen als positiv. Spezifische Antikörper gegen andere Parasitenantigene konnten in den untersuchten Seren nicht nachgewiesen werden.

Schlüsselwörter

Ostmindoro, seroepidemiologische Studie, Toxoplasmose, Bilharziose, Fasziolose, Echinokokkose, Zystizerkose, Trichinose, Toxokarose.

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