our zone. According to these data, and those of other Spanish and European studies [12], a nine-valent vaccine, including serotypes 1 and 5, could be of interest in Spain, as it would increase coverage of children aged < 5 years from 80.2% to 91.7%.

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RESEARCH NOTE

Serological investigation of the prevalence of anti-dengue IgM and IgG antibodies in Attapeu Province, South Laos

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ABSTRACT

The prevalence of dengue antibodies was determined in the Attapeu region of South Laos with 225 blood samples collected from mostly febrile

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patients during the rainy season August – October 2001. An IgM capture ELISA was positive for one (0.4%) sample, while 177 (79%) samples were positive in an indirect IgG ELISA. Of the positive IgG samples, 20 (11.3%) were also positive on blood slides for *Plasmodium falciparum*. Dengue fever seems to be widespread in this area, but clinical dengue diagnosis remains difficult, especially in the first days of illness when physicians have to discriminate between dengue and other febrile illnesses.

Keywords Dengue fever, ELISA, IgG, IgM, Seroprevalence

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Dengue is a mosquito-transmitted arboviral disease, with millions of cases occurring each year [1]. Dengue virus causes a spectrum of clinical manifestations such as dengue fever (DF), a self-limiting flu-like illness with very low mortality, or dengue hemorrhagic fever (DHF). Severe cases with signs of circulatory failure may develop a hypovolaemic shock, termed dengue shock syndrome (DSS), which is associated with a case-fatality rate of >10% [2]. The dengue virus belongs to the family Flaviviridae, with infection caused by four serotypes (DEN-1–DEN-4). Infection in humans with one of the serotypes (primary infection) produces life-long immunity against reinfection with the same serotype, but does not protect against a different serotype [3]. Secondary infection with a different serotype following primary infection is associated with an increased risk of DHF.

A challenging problem with regard to patient management is the rapid differential diagnosis of early symptoms in order to distinguish dengue from other diseases such as malaria. Detection of IgM and IgG antibodies is a useful tool to distinguish between primary and secondary infection. Primary infection is characterised by the presence of significant or rising levels of IgM antibodies in the period 3–5 days after onset of infection, and can persist for 3–5 months. Anti-dengue IgG levels are comparatively low during primary infection, but secondary infection often results in the appearance of high levels of IgG before the IgM reponse. IgG levels rise quickly, peak about 2 weeks after the onset of symptoms, and then decline slowly over 3–6 months. Anti-dengue IgM levels are comparatively low during a secondary infection [3–5]. The objective of the present study was to examine serum samples for anti-dengue IgM and IgG antibodies in the Province of Attapeu in South Laos to provide basic epidemiological data on the situation of dengue infections in this rural area.

From August–October 2001, sera from 225 patients, mostly with a history of fever ($n = 129$), were collected in the Province of Attapeu in South Laos. This region is situated in the south-east part of Lao PDR, close to the border with Vietnam and Cambodia. The study was approved by the ethical committee of the University of Munich, Germany and by the Ministry of Health, Vientiane, Lao PDR. The patients were of ethnic Lao Thung or Lao Loum origin. Inclusion criteria were an age of >1 year, fever or history of fever, and informed consent by the patient or the parents.

Initially, samples from all patients with febrile symptoms were screened with a *Plasmodium falciparum* dipstick test (Paracheck; Orchid, Goa, India). Following a positive dipstick test, thin and thick blood smears for malaria were made, stained with Giemsa by standard procedures, and later read by experienced technicians in the hospitals. The blood samples were collected either in the District Hospital of Xaysettha or in Attapeu Provincial Hospital, allowed to coagulate for some hours at 4 °C, aliquotted into tubes, frozen at −20 °C and later transported to Germany (Department of Infectious Diseases and Tropical Medicine, University of Munich) for further testing. All sera were tested by two different ELISAs (PanBio, Brisbane, Australia) for detection of specific IgM antibodies to dengues–flaviviruses, and for detection of IgG antibodies to the four dengue serotypes. Data were stored in a Microsoft Access database, crosschecked, and then transferred into SPSS v.10 (SPSS Inc, Chicago, IL, USA) for all statistical analyses.

Table 1 summarises the results obtained. The IgM capture ELISA was positive for one (0.4%)

Table 1. Summary of results obtained for 225 serum samples with the ELISAs for dengue IgM and IgG antibodies

	Dengue IgG-positive (n = 177)	**Dengue IgG-negative (n = 48)**
Females (n = 106)	78 (73.6%)	28 (26.4%)
Male (n = 114)	94 (82.4%)	20 (17.6%)
Age (years, n = 224)		
< 10	3 (50.0%)	3 (50.0%)
10–19	32 (62.7%)	19 (37.3%)
29–29	47 (81.0%)	11 (10.0%)
30–39	40 (85.1%)	7 (14.9%)
40–49	29 (82.9%)	6 (17.1%)
> 50	25 (92.6%)	2 (7.4%)
Hospital		
Province (Attapeu, n = 130)	107 (82.3%)	23 (17.7%)
District (Xaysettha, n = 95)	70 (73.7%)	25 (26.3%)
History of fever (n = 129)	101 (78.3%)	28 (21.7%)
No fever (< 37.5 °C)	120 (76.9%)	34 (23.1%)
Fever (> 37.5 °C)	27 (69.2%)	12 (30.8%)
P. falciparum-positive	20 (76.9%)	6 (23.1%)
P. falciparum-negative	156 (78.8%)	42 (21.2%)
Dengue IgM ELISA-positive	1 (100.0%)	
Dengue IgM ELISA-negative	176 (78.6%)	48 (21.4%)

sample from the group aged 10–19 years, while 177 (79%) samples were positive with the indirect IgG ELISA, which indicates a past or recent dengue infection. Of the 130 (57.8%) samples from outpatients in the Province Hospital, 107 were positive in the IgG ELISA. Of the 95 (42.2%) samples from the District Hospital, one was positive for IgM, and 70 were positive for IgG. There was no significant difference in IgG prevalence at the province (82.3%) and district (73.7%) levels. The age distribution was 3–73 years (mean 31 years), with an equivalent gender distribution (female, 48.2%; male, 51.8%). There was no significant difference in the male: female ratio between the seropositive (54.7%: 45.3%) and the seronegative (41.9%: 58.1%) samples. The prevalence of dengue IgG antibodies within the different age groups increased from 50% in the group (n = 6) aged < 10 years to 92.6% in the group (n = 25) aged > 50 years. There was a strong linear association of increasing prevalence with age (Spearman Correlation 0.233, $p < 0.001$; ChiQuadrat 15.4, d.f. = 5, $p < 0.009$). Unfortunately, no paired samples were available to distinguish between primary and secondary dengue infection. In the IgG-seropositive group, 101 patients reported a recent febrile illness (with a fever history of 1–7 days), but only 27 patients had fever of > 37.5 °C at presentation (Table 1). Among all 177 IgG-seropositive patients, 20 were also positive for *P. falciparum*, compared to six of the 48 IgG-seronegative group. In this group, 28 patients reported a history of fever, while 12 patients had a temperature > 37.5 °C at presentation.

This seroprevalence study is, to our knowledge, the first conducted in South Laos, and demonstrated a seroprevalence of 0.4% for anti-dengue IgM and of 79% for anti-dengue IgG. High transmission rates for flaviviruses have been described in South-east Asia. Therefore serological cross-reactivity across the flavivirus group (e.g., Japanese encephalitis virus) is common at the IgG level, and the results must be treated with caution. No previous data on the dengue prevalence situation in the Lao PDR are available, but a similar study from Thailand among children aged ≤ 14 years showed that 32% had anti-dengue antibodies, with 7% of these having a primary and 93% a secondary infection [5]. Studies in Peru and Saudi Arabia have shown an overall prevalence for dengue IgG of 29.5% and 32%, respectively [6,7].

In the present study, a strong linear association of increasing antibody prevalence with age was detected. This may result from a relatively stable transmission rate over decades. This result is in line with previous work that also showed an age-dependent increase of anti-dengue antibodies in exposed populations [6,8,9]. Other results show that dengue prevalence depends on time of exposure, on the risk of getting bitten by an infected mosquito, and thus strongly on age in endemic and in epidemic countries. Higher prevalence rates are often more common in younger age groups during and after dengue epidemics and outbreaks [10].

In endemic areas, misdiagnosis of dengue, Japanese encephalitis or malaria occurs frequently. Awareness and clinical suspicion for patients

with similar illness (fever, headache, vomiting, etc.) are necessary. The present survey demonstrates that dengue fever may be a problem in the region of Attapeu Province. Further detailed serological studies with inclusion of other flaviviruses are recommended.

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RESEARCH NOTE

Serum ferritin levels in West Nile encephalitis

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ABSTRACT

West Nile encephalitis (WNE) presents clinically as aseptic meningitis, meningoencephalitis, encephalitis, or acute flaccid paralysis. Non-specific laboratory findings, e.g., leukopenia and thrombocytopenia, accompany WNE. Lymphopenia is marked and prolonged with WNE. Three patients with WNE were found to have elevated serum ferritin levels. Severity seemed to be directly related to serum ferritin levels. Although preliminary, the results suggested that serum ferritin levels ≥500 ng/mL (normal range 5–187 ng/mL) occur late with WNE, and not in a control group of patients with viral meningitis or encephalitis.

Keywords Encephalitis, ferritin, viral meningitis, West Nile encephalitis

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West Nile encephalitis (WNE) first appeared in the USA in the New York area, and has subsequently spread westwards across the USA, with cases in most states [1–4]. The clinical presentation may be manifested as aseptic meningitis, meningoencephalitis, encephalitis, or acute motor paralysis. The clinical course of WNE presents a spectrum of illness ranging from mild aseptic meningitis to fatal encephalitis. WNE is a diag-

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