

clude that the antibody response in PKDL patients is quite distinct from that in KA patients.

In the past there have been few studies on the host immune response to *L. donovani* antigens in PKDL patients, which would lead to a better understanding of the immunological mechanism and to identification of antigens for use in serodiagnosis. The present study led to identification of 2 antigens of *L. donovani* (110- and 65-kDa proteins) that are recognized with high sensitivity by antibodies in sera from PKDL patients. These 2 antigens need to be purified or produced as recombinant peptides for evaluation, singly or in combination, in more practical assays such as ELISA.

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Short Report

ELISA detection of malaria sporozoites: false-positive results in *Anopheles gambiae* s.l. associated with bovine bloodmeals

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The standard method for detection of malaria sporozoites in vector mosquitoes is dissection and examination of the salivary glands by light microscopy. More recently, an enzyme-linked immunosorbent assay (ELISA) for detection of *Plasmodium* species-specific circumsporozoite protein (CSP) using monoclonal antibodies (MAbs) has been developed (BURKOT *et al.*, 1984). These tests can be easily repeated, carried out independently of the time and place of capture and can distinguish between species of human *Plasmodium*. Many

studies have demonstrated a good correlation between infection rates determined by ELISA compared to salivary gland dissection, with an overestimation of the sporozoite rate by ELISA up to 1.1-1.5 times (SOKHNA *et al.*, 1998). The MAbs have been shown to be highly specific with no or little cross-reaction between *P. falciparum*, *P. malariae* and *P. ovale* (COLLINS *et al.*, 1988). This ELISA has been used in malaria studies in numerous countries. SOMBOON *et al.* (1993), using the ELISA to test pools of mosquitoes captured in animal enclosures in Thailand, reported false-positive results in the detection of sporozoites of *P. vivax* and *P. falciparum* in association with bovine and swine blood. None of the mosquito species tested was known to be a vector of malaria parasites. The reason for the reaction between the MAbs and one or more factors contained in the hosts' plasma remains unidentified. The false-positive results were recorded from the plasma of cow, buffalo and pig but not from human or mouse plasma. A small proportion of blood cell samples also gave false positivity. Dialysable and resistant to heating at 56°C for 30 min, the factor(s) was/were destroyed by 10 min in boiling water. They were not stable in the blood circulation since some cows turned negative or weakly positive when examined 6 months later (SOMBOON *et al.*, 1993; P. Somboon, unpublished data). The authors recommended caution in interpreting results obtained by ELISA, and the use of the dissected head-thorax portion of the mosquito to decrease the risk of false-positive results.

During a 2-year entomological longitudinal study of the transmission of seasonal malaria in the village of Barkedji in the Sahelian region of Senegal, we found, during the rainy season of 1995, a high circumsporozoite

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infection rate of *Anopheles gambiae* s.l. for both *P. malariae* and *P. ovale* in single mosquitoes. The study site has been described by LEMASSON *et al.* (1997). From August to November 1995, 5834 *Anopheles* were collected biweekly by pyrethrum spray collections inside 6 bedrooms. Head-thoraces were tested for CSP by ELISA as described by BURKOT *et al.* (1984), modified by WIRTZ *et al.* (1987) and with MAb 2A10 (*P. falciparum*), MAb 109-179-4 (*P. malariae*) and MAb 110-54-3 (*P. ovale*). The infection rates were 1.20, 0.15 and 0.08% for *P. falciparum*, *P. malariae* and *P. ovale*, respectively. The infection rate for both *P. malariae* and *P. ovale* was 0.32% ($n = 19$). Considering independent infections, the expected number of mosquitoes infected by both *Plasmodium* species ($n = 0.0077$) is significantly different ($P < 10^{-6}$) from the observed number ($n = 19$). This difference suggested false-positive results among mosquitoes positive for *P. malariae* and *P. ovale*.

Bloodmeal sources of a sample of captured female mosquitoes were identified by ELISA (BEIER *et al.*, 1988). Among mosquitoes positive for *P. malariae* alone, or for *P. malariae* and *P. ovale*, a very high frequency of bovine bloodmeals was detected (Table). In an attempt to identify the factors involved in false positivity, we collected bovine blood spots on Whatman filter paper from Barkedji's cows in March 1996. Seventeen (30.4%) of 56 whole blood samples collected from cows (approximately 50 μ L of whole blood eluted in 500 μ L of phosphate-buffered saline) tested by the circumsporozoite ELISA were positive to both *P. malariae* and *P. ovale*, and 4 (7.1%) to *P. malariae* only. Optical densities of positive samples ranged from 0.073 to 1.902 with *P. malariae* and from 0.068 to 0.799 with *P. ovale*. The cut-off levels (twice the average absorbance of negative controls) for *P. malariae* and *P. ovale* ELISA plates were 0.028 and 0.062, respectively. All positive samples were re-examined and results were confirmed. Two weeks later, blood samples from 11 positive cows were collected in tubes containing heparin and Quantitative Buffy Coat tests (LEVINE *et al.*, 1986) and smears were made to search for pathogenic agents. All samples were ELISA tested and CSP positivity was confirmed. Neither *Babesia* sp., nor *Theileria* sp., nor *Plasmodium* sp. was observed; *Anaplasma* sp. was found in one of the cows.

Table. Percentage of indoor-resting mosquitoes fed on cows from August to November 1995 in Barkedji (Senegal), in relation to ELISA results for *Plasmodium*

Result	No. of mosquitoes	Mosquitoes fed on cows (%)
ELISA negative	389	29.6
ELISA positive		
<i>P. falciparum</i>	42	19.1
<i>P. malariae</i>	8	100.0
<i>P. ovale</i>	5	20.0
<i>P. malariae</i> + <i>P. ovale</i>	18	88.9

In our study, ELISA tests gave false-positive results in detection of sporozoites of *Plasmodium* in some dissected head-thoraces of female mosquitoes that had fed on bovine hosts. The bovine blood factor that reacted with the MAbs has not yet been identified. Two hypotheses could explain why those mosquitoes that had fed on positive cows were CSP positive despite only the head

and thorax being used for the ELISA: (a) some blood had remained in the pharynx of the mosquito, or (b) the factor involved can diffuse in haemolymph.

In the light of our results, we endorse the cautionary warning of SOMBOON *et al.* (1993) to those using ELISA to estimate mosquito infectivity rates. It would be advisable to use another method for confirmation of positive results, such as the polymerase chain reaction method described by STOFFELS *et al.* (1995). Another approach would be the preliminary testing of blood samples by CSP ELISA of preferential hosts of the vector populations under study. These precautionary measures should be applied at the beginning of a survey.

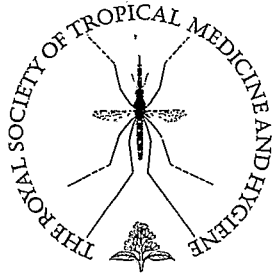
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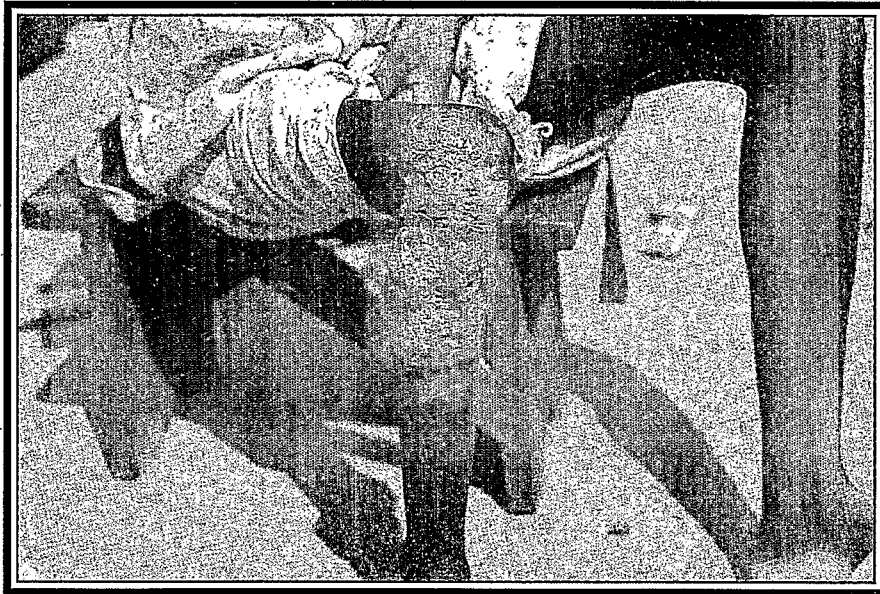
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