FINAL REPORT
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DEVELOPMENT OF METHODS FOR IMMUNODIAGNOSIS
OF HUMAN LIVER FLUKE INFECTION

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Project Duration: July 1986 - December 1990
EXECUTIVE SUMMARY:

Despite minor problems encountered during the research period indicated in the covering page, practically all objectives of the research project have been successfully achieved. Alternative approaches for the detection of *Opisthorchis viverrini* infection in the humans have been proposed, developed and tested. Ten full-length papers have been published in a number of refereed scientific journals and three more are still in press. In addition, three papers have been recently submitted. If all were accepted, there will be a total of 16 papers. Furthermore, there are 17 Abstracts that have been published in the program books of various International and National Conference or Congress. Lastly, the fund available has made it possible for research training of 3 doctoral (Ph.D.) and 3 M.Sc. students.

RESEARCH OBJECTIVES:

The main objective of this research project is to develop simple, sensitive and specific assay(s) for the diagnosis of a human liver fluke infection caused by *Opisthorchis viverrini*. The approach is to characterize, identify and purify relevant antigenic components by appropriate physicochemical and immunological methods using a battery of sera from patients, rabbit polyclonal and mouse monoclonal antibodies. Reasons for the needs to develop these alternative methods have been reviewed in Paper No 1.

RESULTS:

The final accomplishment of the project are presented
in Papers No 12 and 13. Both papers describe the 2 methods that have been developed and results from a limited field testing. Papers No 2 to 11 contain data from detailed studies that are required to develop these 2 methods. Papers No 14 to 16 have been recently submitted for consideration for a publication in various others scientific journals. They describe detailed characterization of the genetic properties of the parasite that pave way for the construction of a specific DNA probe used for the detection of parasite DNA in the stools of patients described in Papers 12 and 13.

In our initial proposal, we mentioned the possibility of a production of relevant antigens by recombinant E. coli carrying gene fragment of Q. viverrini. Despite a number of attempts in various laboratories under the guide of well-qualified investigators, both abroad and within our own faculty, these attempts were not successful. We were able to insert a specific Q. viverrini gene fragment into the E. coli but were not able to make these recombinant bacteria to express the products. Despite these unsuccessful attempts, information obtained was nevertheless important and provided a basis for the construction of a specific DNA probe described in papers 11 to 16.

IMPACT, RELEVANCE AND TECHNOLOGY TRANSFER:

The results achieved in this research project provide 3 alternative approaches for the diagnosis of opisthorchiasis, namely, detection of serum antibody,
detection of soluble antigen in feces and DNA released from eggs expelled with the feces. Although these proposed methods have been tested and compared in a limited field trial, the reliability including particularly sensitivity and specificity has to be tested in a larger field trial which has yet to be started. We are in the process of planning and looking for a future support on this part.

PROJECT OUTPUT:

Lists of publications, meetings attended and graduate students' training are enclosed herewith. As mentioned in the summary on page 2, there will be a total of at least 13 papers published in refereed scientific journals. Three more are in the process and if all are accepted, this will make a total of 16 papers. In additions, there were approximately 17 abstracts of work presented at various local and international scientific meetings. The project also provided opportunity for 3 Ph.D and 3 M.Sc. students to carry on their thesis research.


ORAL/POSTER PRESENTATIONS


6. The characterization of genome and repeated DNA of *O. viverrini*. Same as No.5

7. Analysis of *Opisthorchis viverrini* antigens with emphasis on development of immunodiagnostic assay. XIIth International Congress for Tropical Medicine and Malaria, Amsterdam, Netherlands, 18-23 September, 1988.


10. Development of methods for diagnosis of human liver fluke infection. 30th Year Celebration of the Faculty of Science, Mahidol University, Bangkok, Thailand, 12-14 January 1989.

12. Monoclonal antibodies specific for human liver fluke. 7th International Congress of Immunology, Berlin, Germany. 30 July - 5 August, 1989.


17. Overview of immunology of parasitic infection and alternative approaches to detect liver fluke infection. ASEAN Conference in Medical Laboratory Technology. Bangkok, Thailand, 1-5 April, 1991.
GRADUATE STUDENTS' THESSES

1. Surasak Wongratanacheewin "Characterization of humoral immune response in the serum and bile of patients with opisthorchiasis and its application in immunodiagnosis" (Ph.D. 1987)

2. Rasana Sermswan "Construction of specific DNA probes for Opisthorchis viverrini detection and an attempt to clone genes coding for diagnostic epitopes" (Ph.D. 1990)

3. Sunee Korbsrisate "Cloning and characterization of ribosomal RNA genes of Opisthorchis viverrini (Ph.D., approximately Mid 1991)


5. Sorujsiri Amornpunt "Production and characterization of monoclonal antibodies against metabolic products (excretory secretory antigens) of the liver fluke (Opisthorchis viverrini) (M.Sc. 1989)

6. Teereporn Bureerug "Attempts to identify tumor-associated antigen(s) in cholangiocarcinoma" (M.Sc. 1990)