

PN-ABC-853  
6126

AGENCY FOR INTERNATIONAL DEVELOPMENT  
WASHINGTON, D.C. 20523

DATE: 12/20/88

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18  
FROM: AID/SCI, Victoria Ose *VO*  
SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. 7.240  
PR Aug 88

Attachment

Research Progress Report  
August 1988

Title: Development of a Recombinant Subunit Vaccine for Heartwater in Domestic Ruminants

Grant Number: DPE-5542-G-SS-7073-00

Overall Objective: Develop a safe, effective and economical subunit vaccine that can be used to control heartwater in Africa and other parts of the world.

Specific Aims:

1. Purify C. ruminantium from tick tissues.
2. Construct a C. ruminantium genomic DNA library.
3. Select E. coli transformants expressing C. ruminantium surface proteins.
4. Protect cattle by immunization with lysates of E. coli expressing C. ruminantium surface proteins.

Progress on Specific Aim 1:

The basic isolation procedures and results were given in the March 1988 report.

Progress on Specific Aim 2:

Construct a C. ruminantium genomic DNA library.

- i. Isolate DNA from C. ruminantium isolated from infected nymph midguts.

Midguts were dissected from 4,000 infected nymphs, minced, and sonicated. The sonicated material was centrifuged at 200g and the supernatant collected and centrifuged at 10,000g. The 10,000g pellet was collected and the DNA extracted as we previously described (Barbet AF, et al., Infection and Immunity 55:2428-2435, 1987).

- ii. Purify the unique 20kb DNA band from agarose gels.

Extracted C. ruminantium DNA which contained some tick DNA was electrophoresced on a 0.6% agarose gel and the 20kb DNA band that appears only in preparations of tick nymphs infected with C. ruminantium was isolated. To isolate the 20kb DNA band, it was stained with ethidium bromide, excised from the gel and electroeluted.

- iii. Make a C. ruminantium DNA library in pBR322.

The 20kb DNA fraction was partially digested with restriction endonuclease, Sau3A, to an average size of 3-5kb. The resulting fragments were ligated with Bam HI-cleaved and dephosphorylated pBR322 using T4 DNA ligase. A high-efficiency transformation procedure was used to transform E. coli HB101 to ampicillin resistance. Several colonies with inserts were evaluated for their DNA insert sizes.

Plans for the next 6 months:

- i. Identify E. coli transformants with C. ruminantium specific DNA
- ii. Use the C. ruminantium specific DNA probe to improve the isolation of the organism.
- iii. Make a larger C. ruminantium DNA library in the phage expression vector  $\lambda$ gt11.

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# FINANCIAL STATUS REPORT

(Follow instructions on the back)

1. FEDERAL AGENCY AND ORGANIZATIONAL ELEMENT TO WHICH REPORT IS SUBMITTED Agency for International Development		2. FEDERAL GRANT OR OTHER IDENTIFYING NUMBER DPE-5542-G-SS-7073-00		OMB Approved No. 80-RO180	PAGE OF 1 1 PAGES
3. RECIPIENT ORGANIZATION (Name and complete address, including ZIP code) Washington State University Rm. 240 French Administration Bldg. ATTN: Joan Root Pullman, WA 99164-1025		4. EMPLOYER IDENTIFICATION NUMBER 1-916001108	5. RECIPIENT ACCOUNT NUMBER OR IDENTIFYING NUMBER 11R-3973-1019 & 1020	6. FINAL REPORT <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO	
7. BASIS <input checked="" type="checkbox"/> CASH <input type="checkbox"/> ACCRUAL			8. PROJECT/GRANT PERIOD (See instructions)		
FROM (Month, day, year) September 30, 1987		TO (Month, day, year) December 31, 1990		9. PERIOD COVERED BY THIS REPORT	
FROM (Month, day, year) February 1, 1988		TO (Month, day, year) July 31, 1988			

PROGRAMS/FUNCTIONS/ACTIVITIES ▶	STATUS OF FUNDS						
	(a)	(b)	(c)	(d)	(e)	(f)	TOTAL (g)
a. Net outlays previously reported	\$ 1,008.51	\$	\$	\$	\$	\$	\$ 1,008.51
b. Total outlays this report period	31,913.55						31,913.55
c. Less: Program income credits	-0-						-0-
d. Net outlays this report period (Line b minus line c)	31,913.55						31,913.55
e. Net outlays to date (Line a plus line d)	32,922.06						32,922.06
f. Less: Non-Federal share of outlays	-0-						-0-
g. Total Federal share of outlays (Line e minus line f)	32,922.06						32,922.06
h. Total unliquidated obligations	-0-						-0-
i. Less: Non-Federal share of unliquidated obligations shown on line h	-0-						-0-
j. Federal share of unliquidated obligations	-0-						-0-
k. Total Federal share of outlays and unliquidated obligations	32,922.06						32,922.06
l. Total cumulative amount of Federal funds authorized	150,000.00						150,000.00
m. Unobligated balance of Federal funds	117,077.94						117,077.94

11. INDIRECT EXPENSE	b. TYPE OF RATE (Place "X" in appropriate box) <input type="checkbox"/> PROVISIONAL <input checked="" type="checkbox"/> PREDETERMINED <input type="checkbox"/> FINAL <input type="checkbox"/> FIXED				13. CERTIFICATION I certify to the best of my knowledge and belief that this report is correct and complete and that all outlays and unliquidated obligations are for the purposes set forth in the award documents.	SIGNATURE OF AUTHORIZED CERTIFYING OFFICIAL <i>Linda Boyle</i>	DATE REPORT SUBMITTED August 15, 1988
	b. RATE	c. BASE	d. TOTAL AMOUNT	e. FEDERAL SHARE			TYPED OR PRINTED NAME AND TITLE Linda Boyle, Acct. Supervisor I

12. REMARKS: Attach any explanations deemed necessary or information required by Federal sponsoring agency in compliance with governing legislation.  
SEE ATTACHED

INDIRECT EXPENSE

Rate	Base	Total Amount	Federal Share
43.5%	7,695.44	3,347.53	3,347.53
26.0%	11,981.73	3,115.25	3,115.25
-0-	5,773.60	-0-	-0-
	<u>25,450.77</u>	<u>6,462.78</u>	<u>6,462.78</u>
<b>TOTAL</b>			