PD-ABL-023

FINAL REPORT FOR USAID/CAIRO
COOPERATIVE AGREEMENT no. 263-0152-A-00-2207-00
entitled
CONTROL OF ANAPLASMOSIS AND BABESIOSIS IN EGYPT THROUGH BIOTECHNOLOGY

I. Background

This Project was developed primarily to train a group of Egyptian scientists from the Animal Health Research Institute (AHRI) and from Cairo University in modern methods of biotechnology for application to animal diagnostics development and vaccine development. Two tickborne diseases, anaplasmosis and babesiosis, were chosen as the models with which to work due to the on-going research into these diseases at the University of Florida. A secondary goal of the Project was to train Egyptian scientists from AHRI in innovative non-polluting methods of tick control and specifically our tick-decoy technology. It was anticipated that the Project would provide Egypt with a well-trained nuclei of scientists in veterinary biotechnology and in tick-decoy technology, neither of which existed before the Project.

The proposal for the Project was first developed and submitted to USAID/Cairo as a four-year project in September 1990. The proposal was finally approved for funding in August 1992 as a two-year project (with a six-month no-cost extension through March 1995). This reduction in duration of the Project had a profound effect on the training of the Egyptian scientists, especially when it became apparent that all trainees had to return to Egypt six months before the end of the Project. Consequently, the period of training for each Egyptian scientist had to be reduced markedly with an adverse effect of the level of training that could be accomplished.

The original objectives of the Project were five-fold:

1. To train Egyptian scientists in the use of biotechnology for vaccine development.

2. To train Egyptian scientists in the use of biotechnology for diagnostics development.

3. To allow the Egyptian scientists to utilize the diagnostics in epidemiological surveys to determine the distribution of anaplasmosis and babesiosis in Egypt.

4. To determine the economic impact of anaplasmosis and babesiosis on cattle and buffalo productivity in Egypt.

5. To train Egyptian scientists in the use of pheromone technology for development of innovative methods for tick control in Egypt.

Objectives were pursued with varying degrees of success, with objectives 3 & 4 modified to the design phase only due to the two-year reduction in duration of the Project.

The scientific staff associated with this Project are listed below by institution:

University of Florida

Dr. M.J. Burridge, PI and overall project director.
Dr. D.R. Allred, molecular biologist and supervisor of research and training for babesiosis vaccine development (objective 1).
Dr. A.F. Barbet, molecular biologist and supervisor of research and training for anaplasmosis vaccine development and for anaplasmosis diagnostics development (objectives 1-2).
Dr. J.B. Dame, molecular biologist and co-supervisor of research and training for babesiosis diagnostics development (objectives 2-3).
Dr. M.I. Meltzer, agricultural economist and supervisor of the epidemiological/socioeconomic survey (objectives 3-4).
Dr. R. Reddy G., molecular biologist and co-supervisor of research and training for babesiosis diagnostics development (objectives 2-3).

Old Dominion University, Virginia

Dr. D.E. Sonenshine, pheromone chemist and supervisor of research and training in tick-decoy technology (objective 5).

Animal Health Research Institute, Dokki

Dr. A.F. Abdel-Gawad, co-PI and project director for AHRI.
Dr. M.G.A.M. Aggour, trainee in tick-decoy technology (objective 5).
Dr. M.G. Nessiem, trainee in babesiosis diagnostics development (objectives 2-3).
Dr. A.A.M. Sahlab, trainee in anaplasmosis diagnostics development (objectives 2-3).
Dr. G.H. Salem, trainee in babesiosis diagnostics development (objectives 2-3).
Dr. H. Sobbhy, trainee in tick-decoy technology (objective 5).

Cairo University

Dr. I.M. Reda, co-PI and project director for Cairo University through April 1994.
Dr. M.K. Refai, co-PI and project director for Cairo University since May 1994.
Dr. G. Eid, trainee in anaplasmosis vaccine development (objective 1).
Dr. H.M. Elsaid, trainee in babesiosis vaccine development (objective 1).
Dr. A.A.G. El-Sanousi, trainee in anaplasmosis vaccine development (objective 1).
Dr. R.E.S. Emarah, consultant in epidemiology/economics (objectives 3-4).
Dr. M. Fahmy, parasitologist.
Dr. A.S.H.M. Nassar, trainee in babesiosis vaccine development (objective 1).

II Project Accomplishments

1. Technical/Scientific Accomplishments

a) Objective 1a: Anaplasmosis Vaccine Development

In United States

Assessment was made of three different major surface proteins of *Anaplasma marginale* (MSP1, MSP2, and MSP4) for inclusion in a recombinant vaccine. Significant new information was generated during the Project on all three proteins.

A new construct of MSP1a was prepared in the pET expression vector by Dr. El-Sanousi and tested in a vaccine trial. Although the new recombinant MSP1a was less degraded than constructs produced as fusions in pGEX-2T, the new recombinant appeared less protective to cattle.

The organization and structure of the MSP2 gene family was investigated by Dr. Eid. It was concluded that this polypeptide should not be included in vaccine trials at present because of the large number of alternative different genes comprising this family which indicate a potential mechanism for antigenic variation of this polypeptide. In addition, a new method for preparation of cDNA clones for the expressed MSP2 copies in the Florida isolate of *A. marginale* was developed by Dr. Eid. These clones are currently being analyzed which will give information on the extent of variation within expressed MSP2 genes.

Two vaccine trials including MSP4 have been completed. Significant protection was obtained using recombinant MSP4 or native MSP4 alone, but not when used in combination with MSP1a produced in the pET vector. This is the first evidence for immunoprotection using MSP4.

In Egypt

There was no activity with this objective in Egypt due to the inability of Cairo University to complete construction of its biotechnology laboratories by the end of the Project.

b) Objective 1b: Babesiosis Vaccine Development

In United States

There were three major research aims of this component of the Project: (1) to demonstrate the ability to transfect mammalian cells with *Babesia* genes; (2) to detect expression of transfected genes; and (3) to use expression cloning to identify genes encoding parasite surface-exposed proteins.

The first two aims were accomplished. First, conditions were developed and all parameters optimized to enable high-efficiency transfection of COS7 (green monkey kidney epithelial) cells by electroporation. In addition, procedures were developed to allow highly efficient recovery of transfected DNA back from the COS7 cells to improve the probability of identification of recombinant constructs containing surface-antigen genes. Reporter vectors and shuttle vectors were employed for these experiments, enabling development of a system whereby *Babesia* genes could be inserted into shuttle vectors, libraries created in *E. coli*, recombinant constructs transferred to mammalian cells, and then recovered again in *E. coli*. Second, using the gene encoding the *B. bovis* merozoite surface antigen RAP1 (for **R**hoptry-**A**ssociated **P**rotein; formerly known as Bv60), we have been able to demonstrate through immunofluorescence with monoclonal antibodies the expression of RAP1 in the mammalian cells. Although the overall efficiency of transfection remains relatively low (about 10% of the cells), individual cells expressing RAP1 are easily detected.

Novel information was obtained from this research. The RAP1 antigen used as the model for technique development was found in the parasite both in the anterior organelles and on the surface of the merozoite stage. It was thus assumed that RAP1 could be similarly transported to the surface of mammalian cells; this was found not to be the case. Instead, RAP1 is retained, apparently completely, in the endoplasmic reticulum and golgi apparatus compartments of the COS7 cells. Inspection of RAP1 sequences revealed the presence of a polypeptide sequence KDAL near the carboxy-terminal end of the protein. The sequence KDEL is the canonical sequence used for retention of proteins in the endoplasmic reticulum of mammalian cells. The KDAL sequence may function similarly in the parasite for temporary retention in the rhoptries but may result in permanent retention within COS7 cells. This point will be further pursued by directed mutagenesis, the results of which will have a bearing on the general utility of the proposed cloning method.

Expression by COS7 cells of the exogenous gene encoding the *B. bovis* parasite antigen Bv60 (RAP1) was previously demonstrated by immunofluorescence microscopy with monoclonal antibodies specific to RAP1. Reactivity was restricted to COS7 transfectants that had received recombinant constructs containing the RAP1 gene. During repeats of this experiment it was observed that expression of RAP1 was at least an order of magnitude more common in those transfectants receiving recombinant shuttle vector containing the gene in the appropriate 5'→ 3' orientation relative to the CMV promoter. Attempts to demonstrate the apparent size of the expressed product by immunoprecipitation failed due to excessive background. This background seemed to be caused by association of a low solubility form of RAP1 with detergent-insoluble COS7 components. Additional efforts were not pursued due to a lack of available manpower and funding.

In Egypt

There was no activity with this objective in Egypt due to the inability of Cairo University to complete construction of its biotechnology laboratories by the end of the Project.

c) Objective 2a: Anaplasmosis Diagnostics Development

In United States

The major accomplishment was the training of Dr. Sahlab in new serodiagnostic assays (cELISA and Western blotting) for anaplasmosis. Dr. Eid also received training in the use of DNA hybridization probes.

In Egypt

These assays are currently being used by Dr. Sahlab at AHRI in an attempt to diagnose the presence of anaplasmosis in Egypt, and he has discovered two herds of cattle where most animals are strongly positive in the cELISA. A total of 1,038 serum samples were collected from cattle, sheep, and goats in four localities (Domyat, Zagazig, Giza, and El Fayoum), and 212 were examined for antibodies to *A. marginale* by the cELISA. Eighty-six (41%) of the 212 cattle examined were positive for *A. marginale* antibodies, including 80 (66%) of 122 cattle from El Fayoum.

Dr. Eid at Cairo University collected a total of 250 serum samples from cattle and examined 37. Only three of 17 exotic breeds were found positive for antibodies to *A. marginale*, with all 20 local breeds negative.

d) Objective 2b: Babesiosis Diagnostics Development

In United States

The aim of this component of the research was the detection of as little as one parasite (either *B. bigemina* or *B. bovis*) in a blood sample utilizing a single PCR reaction.

The three genes encoding the major rRNAs of *B. bigemina* were cloned, and the small subunit ribosomal RNA (SSrRNA) was sequenced. Like *B. bovis, B. bigemina* appeared to have three genes encoding the major rRNAs, and our data were consistent with this measurement. PCR amplification of *B. bovis* DNA using universal SSrRNA primers UP1 and UP22 successfully amplified a single 1.65 kb DNA fragment which was cloned. The cloned fragment was sequenced and compared to the *B. bigemina* SSrRNA sequence. An 88.2% sequence identity was observed, and several regions were identified which contained species-specific sequences. This finding was consistent with the sequence structure previously described for the SSrRNA. Using this information, species-specific oligonucleotide probes and PCR primers for *B. bigemina* and *B. bovis* were identified. These two regions were selected based on the location of variable oligonucleotide regions along the SSrRNA suitably spaced for the preparation of species-specific PCR primers to generate a small amplicon. These regions were also selected to contain a variable region suitable for developing a species-specific probe. Different regions of the SSrRNA were targeted for amplification and detection in the two species in order to produce a different-sized amplicon for each, thus allowing multiplex PCR.

While this research was underway, three *B. bovis* SSrRNA sequences were published, one from the Samford isolate obtained in Australia and two from the S stock, a vaccine strain of South Africa. The comparison of all four *B. bovis* sequences in the region selected for development of the diagnostic test demonstrated that the primers were sufficiently conserved for successful amplification of the SSrRNA gene fragment from geographically diverse isolates. Significant diversity was observed in the oligonucleotide region selected as a hybridization probe, JD120. This might have been due to strain differences or the presence of sequence microheterogeneity among the different SSrRNA genes in the *B. bovis* genome. When JD120 was used to probe Southern blots of *B. bovis* genomic DNA, where the three SSrRNA genes are separated by size, all SSrRNA genes of strain Mo7 hybridized with equal intensity. The sequence differences thus appeared to be due to strain differences. To overcome this potential problem for Egyptian isolates, a second detection

probe, JD311, has been selected which is conserved between all four isolates examined. Our data indicated that this probe is equally sensitive to JD120 in detecting the amplified target DNA. Similar strain variability may exist for *B. bigemina*, but there are no data available from other isolates. Strain variation suggested that corresponding sequences from Egyptian strains must be examined to ensure a proper theoretical basis for the application of diagnostic tests in this new location.

This rRNA-based test detected a parasitemia 50 - 500 times lower than that detected using unamplified SSrRNA as a target. The PCR primer pairs and probes detected the homologous Texas isolate of *B. bovis* and the Mexican and Puerto Rico isolates of *B. bigemina*, respectively. The PCR primers and probes were species-specific and did not detect DNA from other hemoparasites of cattle, namely *A. marginale, Trypanosoma brucei brucei*, and *Cowdria ruminantium*.

The rRNA-based test as currently defined meets many criteria for a good diagnostic test. It is relatively simple, fast, and inexpensive. From the low-level parasitemia detected and the specificity of the primers, the test is expected to be sensitive, specific and reliable. The method developed for extracting DNA for PCR is simple and rapid, where extraction of a single sample is completed in less than 2 hr. Forty samples can be extracted in 5 hr (7.5 min/sample). Completion of the test requires 48-72 hr, but may be reduced by using a pressure blotter to transfer the DNA onto a nylon membrane. A limitation on the use of PCR as a diagnostic test is the problem of false-positive results arising from sample-to-sample contamination in the laboratory or from cloned material or PCR amplicons. When precautionary measures were adhered to, the test could be accomplished successfully without false-positive reactions due to carry-over.

The decision was made to apply the newly developed ribosomal RNA-based PCR tests for bovine babesiosis to cattle in Egypt because tests based on the extrachromosomal, apocytochrome b gene sequence were not yet defined. Attempts to define the latter tests involved a study where an extra-chromosomal DNA segment which codes for the apocytochrome b gene of *B. bigemina* was cloned and sequenced. A homologous gene sequence from *B. bovis* was also identified by colleagues at Washington State University. These sequences and the apocytochrome b gene sequence of *Theileria annulata* were compared using genetic computer group sequence analysis programs. Regions conserved among all three species, and genus- and species-specific sequences, were identified. A primer pair was designed from a genus-specific region to amplify *B. bigemina* and *B. bovis* in the same PCR. A

unique oligonucleotide probe was also designed for each species which was able to identify species-specific PCR products. Conditions for PCR amplification and oligonucleotide hybridization for identification of species-specific amplified products were standardized, and the sensitivity and specificity of this PCR method was tested using varying concentrations of purified genomic DNA from *B. bigemina* and *B. bovis*. These probes specifically identified amplified products derived from 5 fg of genomic DNA from the homologous species, and they did not cross-react with amplified products derived from genomic DNA of heterologous species. *B. bigemina* and *B. bovis* organisms, cultivated *in vitro* in bovine erythrocytes, were mixed with normal bovine blood to yield parasites ranging from 1×10^5 to 1×10^1 per ml of blood, and DNA was isolated from all samples and analyzed by this method which detected DNA extracted from as few as two parasites. This method is under further study to demonstrate its practical value as a diagnostic test.

In Egypt

Blood samples were collected from 200 cattle and 150 buffaloes in Upper Egypt (El Moniab abattoir), and genomic DNA was extracted from the blood of 50 cattle. Blood films from all 350 animals were negative for hemoparasites. Ten cattle samples were amplified using the SS rRNA *B. bovis* PCR primers, whereas no amplification was observed with similar *B. bigemina* primers. Also, no amplification was observed in buffalo samples or using common extrachromosomal PCR primers. All the PCR products did not hybridize to all the species-specific oligonucleotide probes designed in the United States and based on the sequence of the American strains. In addition, blood samples have been collected from *Babesia*-infected cattle for isolation and cloning of an Egyptian *B. bigemina* isolate.

c) Objective 3: Epidemiological Survey in Egypt

Essentially no progress was achieved with this objective due to reasons discussed in II.5 below.

d) Objective 4: Economic Impact

Again, no progress was achieved with this objective due to reasons discussed in II.5 below.

e) Objective 5: Tick Decoy Development

In United States

The cholesteryl esters that comprise the mounting sex pheromone of the camel tick *Hyalomma dromedarii* were extracted and successfully identified. A total of six different cholesteryl esters were identified: cholesteryl acetate, cholesteryl laurate, cholesteryl linoleate, cholesteryl oleate, cholesteryl palmitate, and cholesteryl stearate. In addition, each of the fractions containing these different compounds was shown to have biological activity when challenged with heterospecific males in a pheromone bioassay. Furthermore, each of the authentic cholesteryl esters was shown to have strong biological activity when challenged with both conspecific and heterospecific males in the same bioassay.

Collections of the cattle tick *Boophilus annulatus* were obtained from Egypt and of the tick *B. microplus* from the U.S. Department of Agriculture in Mission, Texas. Preliminary analysis did not reveal any evidence of cholesteryl esters, but it did reveal evidence of cholesterol. The sex pheromone of *B. annulatus* ticks was not identified, but it is known to be different from that of *H. dromedarii* ticks since cholesteryl esters were absent and field tests with cholesteryl ester-coated decoys failed to excite pheromone responses in *B. annulatus* ticks. This discovery that the chemical composition and behavioral responses of *B. annulatus* are very different from those of other metastriate ixodid ticks is an important finding.

With the successful identification of the mounting sex pheromone of *H. dromedarii* ticks, we were able to formulate the mixture of cholesteryl esters and to coat the decoys with this mixture so as to simulate the natural pheromone of the camel tick.

During his training at Old Dominion University, Dr. Aggour made about 2,000 tick decoys impregnated with cyfluthrin and 2,6-dichlorophenol and coated them with the mounting sex pheromones of *H. dromedarii* ticks. These decoys were prepared for testing in Egypt in 1994.

After the Egyptian trainees returned to Egypt, work continued at Old Dominion University which led to (1) the discovery of improved adhesives that enhanced the likelihood of long-term adhesion of the decoys; and (2) the development of improved methods for coating the

plastic surfaces of the decoys to bond the cholesteryl esters and extend the duration of the mounting sex pheromone activity.

In Egypt

Field tests to evaluate the efficacy of these tick decoys were conducted on camels in Egypt. The decoys contained the pesticide cyfluthrin in the first test and diazinon in the second. In the first trial, female ticks either died without oviposition or laid infertile eggs, presumably because they were never inseminated. The decoys effectively attracted and killed many of the ticks and completely disrupted mating, clearly demonstrating the success of the decoy technology using cyfluthrin in control of *H. dromedarii* ticks on camels. The second trial was not successful, but it did demonstrate that cyfluthrin is a much more preferable pesticide than diazinon for camel ticks.

2. Human Resource Development

The following Egyptian scientists received training through the Project:

Name	Location of Training	Dates of Training						
Dr. Sobbhy	Old Dominion University	1	Nov.	1992	-	1	June	1993
Dr. Salem	University of Florida	2	Nov.	1992	-	29	Oct.	1993
Dr. Eid	University of Florida	2	Nov.	1992	-	21	Oct.	1993
Dr. Elsaid	University of Florida	1	Dec.	1992	-	14	Aug	1993
Dr. Nessiem	University of Florida	5	July	1993	-	4	Mar.	1994
Dr. Aggour	Old Dominion University	12	July	1993	-	21	Jan.	1994
Dr. El-	University of Florida	12	July	1993	-	21	Jan.	1994
Dr. Nassar	University of Florida	18	July	1993	-	6	Feb.	1994
Dr. Sahlab	University of Florida	13	Sep.	1993	-	6	Mar.	1994

The training of Drs. Eid, Elsaid, El-Sanousi, and Nassar was the essential component of objective 1 (training in vaccine development technology). The training of Drs. Salem, Nessiem, and Sahlab was the essential component of objective 2 (training in diagnostics development technology) and an important component of objective 3 (utilization of diagnostics in an epidemiological survey). The training of Drs. Sobbhy and Aggour was the essential component of objective 5 (training in pheromone technology).

Three of the Egyptian trainees made official trips while in the United States:

a) Dr. Eid: He attended the 12th Annual Meeting of the American Society for Virology in Davis, California, from 10-14 July 1993, and then visited

the laboratory of Dr. Tilahun Yilma at the University of California (Davis) from 15-24 July 1993. This trip helped Dr. Eid gain virological perspectives on vaccine development technology (objective 1).

b) Dr. Salem: He visited St. Croix in the U. S. Virgin Islands from 1-31 August 1993. This trip provided Dr. Salem with the opportunity to learn how to handle field samples destined for use in the PCR-based diagnostic tests and provided the babesiosis samples necessary to complete his training and to initiate Dr. Nessiem's training (objective 2). Once Dr. Salem had evaluated his diagnostic test with the field samples collected on St. Croix, it was anticipated that, upon his return to Egypt, he would utilize the test in the epidemiological survey (objective 3). St. Croix was selected for Dr. Salem's trip because it is the nearest U.S. territory to Florida where bovine babesiosis is endemic.

c) Dr. Nessiem: He visited the laboratories of Dr. William Todd and Dr. Jack Malone at Louisiana State University in Baton Rouge from 24 January 1994 to 7 February 1994. This trip provided Dr. Nessiem with the opportunity to learn about the serodiagnostic methods being performed in those laboratories, broadening his training experience (objective 2).

Five U.S.-based faculty involved with the Project made official trips to Egypt:

a) Drs. Burridge and Barbet: They visited Egypt from 27 June 1992 to 2 July 1992 to initiate the Project and to interview candidates for biotechnology training at the University of Florida (objectives 1 & 2).

b) Dr. Sonenshine: He visited Egypt from 6-9 September 1992 to interview candidates for pheromone training at Old Dominion University (objective 5).

c) Dr. Dame: He visited Egypt from 24 April 1994 to 6 May 1994 to assist in the establishment of the biotechnology laboratories at AHRI and to assist Egyptian scientists in initiating their research program at AHRI (objective 3).

d) Dr. Reddy: He visited Egypt from 11-20 May 1994 to follow-up on Dr. Dame's visit and to provide guidance on continued research at AHRI (objective 3).

e) Drs. Burridge and Reddy: They visited Egypt from 13-17 March 1995 to close out the Project (Burridge) and to complete transfer of diagnostic technologies to AHRI (Reddy).

3. Facility Improvement

Two sets of biotechnology laboratories were established, one by renovations at AHRI and the other by new construction at Cairo University. In addition, animal-housing facilities were constructed at both institutions. By the end of the Project, only the laboratories at AHRI were functional.

The following items of equipment were purchased for delivery to AHRI and Cairo University:

Quantity and item		Date of Delivery in Egypt
2	autoclaves	5 November 1993
2	water purification systems	5 November 1993
2	electronic balances	5 November 1993
4	Harvard trip balances	5 November 1993
2	circulating coolers	5 November 1993
2	drying ovens	5 November 1993
2	SpeedVac systems	8 November 1993
2	orbital shakers	19 November 1993
2	rocker platforms	19 November 1993
2	high-speed centrifuges	23 November 1993
2	table-top centrifuges	23 November 1993
4	microfuge centrifuges	23 November 1993
4	rotor assemblies	23 November 1993
12	rotors	23 November 1993
2	quickseal tube kits	23 November 1993
2	rotor tube kits	23 November 1993
2	ultracentrifuges	23 November 1993
2	peristaltic pumps	6 December 1993
2	notebook computer systems	8 December 1993
2	ELISA readers	22 December 1993
2	Kineti-calc software systems	22 December 1993
2	power supplies	25 December 1993
4	sequencing gel systems	25 December 1993
2	film processors	25 December 1993
2	continuous flow chambers	22 January 1994
2	cup horns	22 January 1994
2	sound enclosures	22 January 1994
2	ultrasonic cell disrupters	22 January 1994
2	autoexposure camera systems	8 February 1994
1	inverted microscope	8 February 1994
3	standard microscopes	8 February 1994
2	phase & flourescent microscopes	8 February 1994
4	bio-dot apparatuses	23 February 1994
2	CHEF systems	23 February 1994
2	gel-drying systems	23 February 1994
1	mini-protean cell	23 February 1994
4	multi-channel pipets	23 February 1994

Quantity and item		Date of Delivery in Egypt
2	power supplies	23 February 1994
1	tube cell	23 February 1994
2	vacuum blotters	23 February 1994
2	chest freezers	7 March 1994
2	hybridizers	7 March 1994
2	ice makers	7 March 1994
2	inventory systems	7 April 1994
2	ultra-low freezers	7 April 1994
4	explosion-proof refrigerators	7 April 1994
2	isotemp incubators	11 May 1994
2	shaker floor incubators	11 May 1994
2	microcentrifuges	11 May 1994
2	pH meters	11 May 1994
2	universal platforms	11 May 1994
2	spectrophotometers	11 May 1994
2	UV strata linkers	11 May 1994
2	vacuum pumps	11 May 1994
4	water baths	11 May 1994
2	tempcycler microtube incubators	17 July 1994
2	fraction collectors	17 July 1994
2	peristaltic pumps	17 July 1994
2	UV detectors	17 July 1994
2	upright freezers	17 July 1994
2	timed sequence incubators	17 July 1994
2	safety cabinets	17 July 1994
3	cryogenic freezers	17 July 1994
2	transilluminators	17 July 1994
2	gel documentation systems	17 July 1994
2	gelbases	17 July 1994
2	image stores	17 July 1994
4	computers	8 August 1994
2	laser printers	8 August 1994
1	Geneamp system	9 May 1994*
1	Millenium chromatography pump	27 May 1994*
1	desk jet printer	27 May 1994*
2	HPLC pumps	25 October 1994*
1	fraction collector	25 October 1994*
1	gradient controller	25 October 1994*
1	stirrer	14 March 1995*
1	orbital shaker	14 March 1995*
1	rocker platform	14 March 1995*
1	platform rocker	14 March 1995*
1	protean cell	14 March 1994*
3	power supplies	14 March 1995*
2	transblot cells	14 March 1995*
1	pulseware switcher	14 March 1995*
1	laserjet printer	14 March 1995*
1	DNA proscan system computer	14 March 1995*

Quantity and item		Date of Delivery in Egypt
3	balances	14 March 1995*
1	pH meter	14 March 1995*
2	microcentrifuges	14 March 1995*
1	microfuge	14 March 1995*
1	storage refrigerator	14 March 1995*
1	storage freezer	14 March 1995*
1	fluorometer	14 March 1995*
1	X-ray film processor	14 March 1995*
1	pipetman pipette	14 March 1995*
2	hybridization ovens	14 March 1995*
1	microscope items	14 March 1995*
1	universal injector	24 March 1995*

*Date shipped from U.S.

These items of equipment were essential to establish the biotechnology diagnostic laboratory and the tick decoy laboratory at AHRI and the biotechnology vaccine-development laboratory at Cairo University.

4. National/International Relationships Developed

The primary international relationships that have developed during the Project have been between the two U.S. institutions and the two Egyptian institutions. Good working relationships have developed at the scientist level between the University of Florida and the Animal Health Research Institute, relationships which were reinforced by the visits of Dr. Dame and Dr. Reddy to AHRI in April 1994, in May 1994, and in March 1995.

At the national level, this Project has strengthened further an already very strong professional relationship between the University of Florida and Old Dominion University in the area of innovative methods for tick control.

5. Problems Encountered & Solutions Found

Several problems were encountered during this Project:

a) Time limitations: A major problem was the compression of the Project from a four-year to a two-year Project, primarily because of delays in funding. This problem was further compounded by the requirement for early return of trainees to Egypt. These factors meant that the training period for the nine trainees was reduced from four to 1.5 years. The problems were overcome , albeit not optimally, by modifying the Project with reduced periods of training. This solution has had its drawbacks, most importantly leaving the Egyptian scientists with, in most cases,

inadequate training to apply their knowledge in Egypt without further guidance from scientists in the United States. Finally, the Project was given a six-month no-cost extension through March 1995.

b) Premature departure of trainees: The time-limitation problems outlined above were further complicated by the early departure of five of the nine trainees. Three of the four trainees in vaccine development technology (Dr. Elsaid, Dr. El-Sanousi, and Dr. Nassar) returned to Egypt earlier than planned, and both trainees in pheromone technology (Dr. Aggour and Dr. Sobbhy) did too. These premature departures compromised their training programs and disrupted the research plans.

c) Trainee stipends: All nine trainees were paid inadequate stipends, placing hardships both on the trainees themselves and on their families back in Egypt. This problem had a very detrimental impact on the morale of the trainees and undoubtedly resulted in at least some of them returning to Egypt prematurely. Attempts to increase the stipends proved fruitless.

d) Administration of trainees: All trainees were administered through the San Diego State University Foundation. Everyone, Egyptian and American alike, at the University of Florida and at Old Dominion University were less than happy with this arrangement. The trainees perceived, rightly or wrongly, that the Foundation had little interest in them, and this exacerbated the problems associated with inadequate stipends for the trainees. No solution was found to this problem, but it would undoubtedly be preferable in future if trainees are administered by the same institution that supervises their training program.

e) Removal of trainees from Project: Three of the trainees did not participate in Project activities after their return to Egypt; Dr. Elsaid was excluded from the Project by Cairo University, Dr. El Sanousi left to work in Kuwait, and Dr. Nessiem emigrated to Australia. This loss of trained manpower in Egypt has had and will continue to have a detrimental impact on the establishment of research activities related to the Project in Egypt, especially at Cairo University.

f) Communications: A significant problem to achievement of Project objectives, especially in the latter stages, has been poor communications. Particularly noticeable has been the poor communication between the trainees and their American counterparts after the trainees returned to Egypt, making it very difficult for the U.S. collaborators to assist them in the application of their training. Solutions to this problem were to be discussed at the final TAG evaluation of the Project in August 1994, but

these discussions never materialized due to the postponement of the evaluation. These communications problems continued to the end of the Project.

g) Involvement of trainees in vaccine trials: The communications problem mentioned above was further exacerbated by the abrupt cancellation of Dr. Eid's return visit to the University of Florida to assist in the anaplasmosis vaccine trial. This action meant that no Egyptian trainee had any practical experience with the testing of vaccines developed using biotechnology.

h) Establishment of laboratories in Egypt: The laboratories at AHRI and at Cairo University were not functional when the trainees returned to Egypt, delaying their ability to apply their new knowledge. At the end of the Project, the laboratories at Cairo University were still non-functional.

i) Transportation in Egypt: The University of Florida was to have purchased two vehicles for Egypt. However, their purchase was postponed by USAID/Cairo and then transferred to the local currency budget. Recently authority to purchase the vehicles was cancelled. The resultant lack of vehicles for the Project has greatly hampered field work within Egypt.

j) Inability to hire Egyptian consultant: Funds were available to hire an Egyptian consultant in epidemiology/economics to provide the essential local input into the development and eventually the carrying out of an epidemiology/economics survey for anaplasmosis and babesiosis in Egypt. However, approval was not granted to hire the selected consultant, Dr. Emarah, effectively halting our efforts to proceed with objectives 3 and 4 of the Project. Consequently, we had no alternative but to cancel objectives 3 and 4 without any meaningful progress made.

III. Financial Report

1. U.S. Dollar Budget and Expenditures

As of 1 March 1995, 93% of the US$ budget had been expended:

	Original budget	Revised budget	Funds expended through 2/28/95	Projected balance at end of Project
Direct labor	$ 154,719	$ 201,290	196,698	$ 648
Travel	32,370	25,478	16,911	549
Equipment	656,269	672,162	670,502	0
Vehicles	39,684	0	0	0
Supplies	96,888	190,538	187,475	0
Freight	65,627	39,951	30,651	0
Experimental animals	154,014	52,319	52,319	0
TAG	48,275	70,983	27,403	6,290
Consultants	21,060	0	0	0
Publications	12,000	2,222	0	0
Subcontract	115,773	98,855	95,557	0
Other direct costs	32,887	58,736	56,736	775
Total Direct Costs	**$1,429,566**	**$1,412,534**	**S1,334,252**	**$ 8,262**
Indirect costs	286,355	303,387	255,167	8,274
TOTALS	**$1,715,921**	**$1,715,921**	**$1,589,419**	**$ 16,536**

IV. A Look to the Future

Economic Impact of Project

The economic impact of the Project to Egypt should have been substantial if the Project had not been terminated in March 1995. For about $2,000,000 in USAID investment, the following outputs have already been realized:

1. Two sets of biotechnology laboratories have been established in Egypt, one for diagnostic work at AHRI and one for vaccine development at Cairo University, and both sets have been supplied with the most modern biotechnology equipment.

2. A pheromone laboratory has been established in Egypt at AHRI and supplied with the most modern chemical equipment.

3. Seven Egyptian scientists, four from Cairo University and three from AHRI, have been trained in modern techniques of biotechnology at the University of Florida and four have returned to Egypt to work on the Project.

4. Two Egyptian scientists from AHRI have been trained in modern techniques of pheromone chemistry at Old Dominion University and both have returned to Egypt to work on the Project.

5. The groundwork has been laid for development of the tick-decoy technology in Egypt for the control of ticks of livestock, with the potential prospect of commercialization of this technology through the private sector in Egypt.

However, for the full economic impact of this Project to be achieved, programs in the laboratories must be developed by the Egyptian trainees, with on-going assistance from the United States, and the tick-decoy technology must be adequately tested and adapted to Egyptian conditions, again with on-going assistance from its inventors in the United States. How this will occur now that the Project has been terminated is unknown.

Scientists at the Egyptian institutions have made preliminary estimates of the impact of anaplasmosis, babesiosis, and their tick vectors on the Egyptian economy. The main parameters are unknown at this time. Therefore, the study approximated the infected population based on a 1986 survey conducted by the Government Office of Veterinary Services. The losses on both the macro and micro levels was calculated for different price parameters. The losses in meat were estimated at LE 147,000,000. The losses in milk production differed in acute, subacute and chronic cases. The range of losses, such as from reduced hide quality, reduced milk yield, infertility, and unthrifty calves, was estimated at LE 141,000,000. These estimates showed that national losses from the tickborne diseases anaplasmosis and babesiosis were in the range of LE 383,000,000-LE 440,000,000, with the mean estimated national loss at about LE 412,000,000. At the individual animal level, the losses were estimated at LE 1,230 per head, including costs of veterinary care, with the maximum loss in acute cases estimated at LE 4,000 per head.

2. Aspects Left Undone and Plan of Action

a) Anaplasmosis Vaccine Development: The analyses of MSP2 cDNA clones prepared by Dr. Eid and publication of the second part of Dr. Eid's project were not completed by the end of Project. Plans have been made to try to complete and publish these studies in collaboration with colleagues at Washington State University. Publication of Dr. El Sanousi's data will also be completed soon.

b) Babesiosis Vaccine Development: The creation of *Babesia* libraries and immunoscreening in mammalian cells was not completed by the end of the Project. In addition, Egyptian isolates of *Babesia* species were not made by Cairo University scientists.

c) Anaplasmosis Diagnostics Development: Egyptian isolates of *A. marginale* were not made by AHRI scientists.

d) Babesiosis Diagnostics Development: The lower limits of detection and reproducibility of PCR-based tests for babesiosis need to be compared, specifically the test based on the SSrRNA gene of nuclear DNA and the similar test based on the apocytochrome b gene of extrachromosomal DNA, using North American isolates of *B. bovis* and *B. bigemina*. Then the tests which reproducibly detect the smallest number of parasites must be selected and adapted for use with Egyptian isolates. The sensitivity and specificity of the selected tests must be determined for detection of *B. bovis* and *B. bigemina* strains native to Egypt.

e) Epidemiological Survey in Egypt: Work on this objective of the Project hardly began, having never gone beyond identification of the scientists to be involved, namely Dr. Meltzer of the University of Florida and Dr. Emarah of Cairo University. Permission to hire Dr. Emarah was denied by USAID/Cairo.

f) Economic Impact of Diseases in Egypt: Similarly, work on this objective barely began, with only Dr. Meltzer and Dr. Emarah identified as the economists to become involved.

g) Tick-Decoy Development: Identification of the pheromone of *B. annulatus* ticks and formulation of the decoys containing the pheromone were not completed by the end of the Project. However, Egypt remains an ideal venue for identification of *B. annulatus* pheromones and development of decoys to control these ticks. Consequently, we have submitted a preproposal to NARP at its request for continuation of commercial development of the attractant decoy technology in Egypt.

3. New Problems/Opportunities Discovered and Plan of Action

New opportunities that have arisen during this short Project are opportunities for collaboration in animal biotechnology research and in tick-decoy development (and its potential commercialization) through the establishment in Egypt of laboratories furnished with modern high-technology equipment. Furthermore, these laboratories are staffed by Egyptian scientists well trained in the United States and eager to utilize their newly acquired technical knowledge. As indicated above, if the tick-decoy technology maintains its early promise, there may be opportunities for involvement of the private sector in commercialization of this technology in Egypt. These opportunities created by a short Project of only two-year's duration are indeed encouraging, but if we are to take advantage of these opportunities it would seem prudent for USAID to extend the Project from the past training and building phase into a new collaborative and production phase.

V. Annotated Bibliography

1. Workplans

Workplans were prepared at the University of Florida and submitted to USAID/Cairo for each of the two years of the Project. Each workplan contained the following: objectives for anaplasmosis vaccine development, objectives for babesiosis vaccine development, objectives for diagnostics development, objectives for tick-decoy development, plans for training program by trainee, relationship of activities to outputs and research priorities, expected progress by end of year, compliance with biotechnology regulations, information dissemination activities, and projected budget.

2. Program Performance Reports

Program performance (progress) reports were prepared at the University of Florida and submitted to USAID/Cairo for every quarter of the Project (i.e., eight quarters in all over two years). Each report summarized the progress made with Project objectives during that quarter. In addition to the program performance reports, quarterly progress reports were prepared by the Egyptian co-PIs. The Egyptian reports included such information as trip reports of Egyptian scientists, research activities in Egypt, progress of Egyptian trainees in the United States, procurement of animals, equipment and supplies in Egypt, data on status of equipment purchased in the United States, and fiscal data on the local-currency budget.

3. Training Reports

Training reports were prepared at the University of Florida and submitted to USAID/Cairo for every quarter of the Project in which training activities occurred (i.e., the first seven quarters of the Project). Each report gave details of the training activities during that quarter by trainee.

4. Annual Activity Reports

Annual activity reports were prepared at the University of Florida and submitted to USAID/Cairo for each of the two years of the Project. Each annual report summarized the progress made during that year in accomplishment of the objectives set forth in the annual workplan and, in addition, gave an impact analysis report and an expenditure report.

5. Mid-term Report to Technical Advisory Group

A detailed report was prepared at the University of Florida for the Technical Advisory Group (TAG) prior to the TAG's mid-term evaluation of the Project. The report gave details of progress in training and research as well as of budgetary analyses and data on equipment purchased for Egypt.

6. Mid-term and Final Reports from Technical Advisory Group

Following its mid-term evaluation of the Project in Gainesville, Florida, in January 1994, the TAG wrote a mid-term report on the Project and submitted it to USAID/Cairo. The report contained reviews of each aspect of the Project's work (i.e., training and technology transfer, vaccine development, diagnostics development, epidemiological and economic studies, and pheromone-based tick control methods), a review of the budget, and recommendations for future actions to be taken by the Project. The final TAG evaluation of the Project took place in Florida in March 1995, and a final TAG report is awaited.

7. Trip Reports

Trip reports were prepared by each U.S.-based traveller and submitted to USAID/Cairo for each official trip made to Egypt as documented in section II.2 above. Each report summarized the activities to the traveller while on the trip, including identification of all individuals met on official business.

8. Publications in Scientific Journals

The following two papers on Project research have been published in international refereed journals:

a) Sobbhy, H., Aggour, M.G., Sonenshine, D.E., & Burridge, M.J. Cholesteryl esters in the body surfaces of the camel tick, *Hyalomma dromedarii*(Koch, 1844) and the brown dog tick, *Rhipicephalus sanguineus* (Latreille, 1806). Exp. Appl. Acarol. 18:265-280, 1994.

b) Palmer, G.H., Eid, G., Barbet, A.F., McGuire, T.C., & McElwain, T.F. The immunoprotective *Anapalasma marginale* major surface protein 2 is encoded by a polymorphic multigene family. Infect. Immun. 62:3808-3816, 1994.

Several other manuscripts are in preparation and hopefully will be published later this year.

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