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Colonization and Containment of Hyalomma Marginatum Rufipes For Studies on the Transmission of Crimean-Congo Hemorrhagic Fever

Final Report

Daniel E. Sonenshine, Ph.D.

July 31, 1987

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-86-C-6169

Old Dominion University Research Foundation
P.O. Box 6369
Norfolk, Virginia 23508

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This report describes services for safe handling, colonization and containment of exotic African ixodid ticks at the Disease Assessment Division, USAMRIID, Fort Detrick, Frederick, MD. Diagrams illustrate facilities to be designed, equipment to be fabricated and record keeping forms to be generated. Techniques for tick feeding, colonization of tick species and related matters are described. Training in tick-rearing procedures is also described. Finally, a literature library with over 700 pertinent references was provided with procedures for sorting by author, journal, subject or other desired need.

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FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

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I. STATEMENT OF THE PROBLEM

The purpose of this contract was (1) to provide the assistance and scientific expertise needed to facilitate the colonization of exotic African tick species, especially Hyalomma marginatum rufipes and other Hyalomma marginatum subspecies; and (2) to insure the proper containment of these man-biting tick vectors so that they could be used safely to study the mechanisms of transmission of Crimean-Congo Hemorrhagic Fever (CCHF).

To meet these needs, protocols were planned and discussed with USAMRIID personnel. The protocols for colonizing exotic African ticks assumed the following:

(1) a colony of each species was to be created and maintained in such a manner as to provide all necessary specimens for transmission experiments without risk of escape. Containment was regarded as an absolute necessity;

(2) the colony was to be created and maintained at USAMRIID, Fort Detrick, MD. no other sites were to be used;

(3) ticks from the stock colony would be infected and housed in the P-3 infectious area ("hot" area) independent of the stock colony and destroyed when the experiments are completed;

(4) a trial program will be established with a native American species, e.g., Dermacentor variabilis (Say) to verify the reliability of the containment procedures. This trial program will be completed in 6 months;

(5) a special room would be designated for the feeding of exotic ticks on hosts, while special incubators, transport devices, and containment equipment would be fabricated as needed.

(6) log books and data forms would be used by the colony maintenance personnel to document the status of each species colonized and the fate of the specimens. When possible, this recordkeeping work would be computerized;

(7) to support the work on colonization of exotic African ticks, especially Hyalomma marginatum rufipes, and transmission of CCHF, a review of the literature and a computerized literature data base is needed.

II. BACKGROUND

The basic etiology, epizootiology and epidemiology of Crimean-Congo Hemorrhagic Fever (CCHF) has been the subject of intensive study in recent years. Undoubtedly, the most extensive and thorough review of this subject is that of Hoogstraal (1979). CCHF virus is widespread over vast regions of the Paleartic, Oriental and Ethiopian faunal region, predominantly in steppe, savannah, dry deciduous forest and other semi-arid biotypes favored by its ixodid tick vectors. In Africa, CCHF is enzootic from Senegal, Nigeria, The Central African Empire and Zaire in the western part of the continent eastward to Kenya. Tanzania and Ethiopia in east Africa (Hoogstraal, 1979). Among the various ixodid vectors in tropical Africa, 2 are especially important, Hyalomma marginatum marginatum Koch, 1844 and Hyalomma marginatum rufipes Koch, 1844 (= H. rufipes). The former is established in the Crimea, Astrakhan and other republics of the Soviet Union as well as in southern Europe. H. m. marginatum is transported to Africa with migratory birds, where it also survives in the Mediterranean climate zones of North Africa (Hoogstraal, 1979). H. m. rufipes is established predominantly in the Ethiopian faunal region, but its range extends to areas of Soviet Central Asia (Hoogstraal et al. 1961). In Africa, its range extends from the South African highlands northward to the Nile River Valley of Egypt and across the Red Sea to scattered localities in Yemen and southern Saudia Arabia. This subspecies, like its close relative, H. m. marginatum, is also transported across vast distances by the agency of migratory birds. In Ethiopia, where the tick is widespread, warm but moderately dry lowlands are considered optimum for H. m. rufipes (Pegram et al. 1981). Further south, in Kenya, Somalia, Tanzania and Zambia, it is distributed in regions where the annual rainfall ranges from 250-875 mm/year.

According to Hoogstraal (1956), its range in Africa is limited to regions with annual rainfall between "ten to thirty inches a year" or "where a long, severe dry season occurs between an annual rainy season of approximately forty inches."

Hosts for the Hyalomma marginatum complex include, predominantly, migratory birds, hares, and hedgehogs for the immatures, and various domestic herbivores for the adults. Adults may also feed on dogs, cats, and even humans (Hoogstraal, 1979). Hoogstraal et al. (1963) report that adults also feed on hares. According to Hoogstraal (1956) this tick assumes a 2-host feeding pattern when allowed to parasitize hares.

There is little evidence of H. marginatum seasonal activity in Ethiopia, at least not in the studies done in that country (Pegram et al. 1981). However, this is not the case elsewhere. In Bulgaria, larval and nymphal H. m. marginatum exhibit a well defined seasonal activity period, from July to October. The question of seasonal activity in this species, especially in the African subspecies, merits further investigation.

The biology of this tick in the laboratory is poorly known. Hoogstraal (1956) cites studies by Theiler, showing a life cycle of 4-5 months, but details of hosts, incubator temperatures, and relative humidities were not given. Optimum temperatures and relative humidities for melting, oviposition and hatching of H. m. rufipes are unknown.

This limited review provides the basis for the proposed work, and also reveals the difficulties that were expected in the colonization of H. marginatum subspecies and also other exotic African ticks. However, there was reason to anticipate success in feeding the ticks on laboratory hosts, e.g. rabbits and guinea pigs. Consequently, the limited data available offered optimism in attempting colonization of these species.

Containment procedures for housing tick vectors are generally not available in published documents. Consequently, these procedures had to be developed de novo. Therefore, this document can serve as a convenient model for future use by government agencies planning studies with exotic arthropod vectors.

III. RATIONALE

The rationale was to formulate procedures for (1) housing the ticks in various life stages under strict isolation, (2) transporting ticks for feeding in transport devices that would prevent inadvertent spread in the event of accidents, (3) feeding of ticks on laboratory animals on a specially designed table to minimize escape of ticks during feeding, (4) renovation or modification of the isolation room to exclude escape of any ticks that escaped from the feeding table, and (5) use of native American ticks (uninfected, from a laboratory colony) to test the containment procedures.

When the preliminary stages noted above had been achieved, travel was planned to various countries in Africa to acquire the exotic tick species needed for the research. Upon return, colonization was to be initiated.

Specific personnel were to be designated as responsible for the management of the tick colonies. To facilitate their supervision of this resource and further reduce the risk of accidental escape of exotic ticks, record-keeping forms and log books were planned. Eventually, these would be computerized to facilitate retrieval of information and generate status reports.

A literature data base was recommended in order to provide a knowledge base for questions concerning the biology of these exotic species and their role in the epidemiology of Crimean-Congo Hemorrhagic Fever.

IV. METHODS

The following is a synopsis of methods that were proposed for the performance of these studies. A more detailed description is contained in the original proposal.

The entire stock colonies of exotic tick species were to be maintained in the insectary at USAMRIID, Fort Detrick, MD.

1. Facilities

All specimens were housed in a special area of the insectary which consisted of two adjoining rooms. One room was to contain a specially-designed infestation table. The main room of the insectory contained the incubators where the ticks were housed during the non-feeding stages.

2. Personnel

Qualified laboratory personnel were indoctrinated into the hazards of uncontrolled tick infestations, safety, precautions, the importance of accurate recordkeeping (including accounting for all vials, hosts, treatments and even all adult ticks), log books for recording data, handling of vials, specimens and animals, and personal hygiene. The colony manager was asked to provide status reports on a regular basis. These were to detail the numbers and physiological stage of all life stages, numbers of vials, infestations in progress, and all other aspects regarding the state of the colony. The colony manager was expected to provide specimens for future experiments.

3. Procedures

Specimens were held in gauze covered vials in the incubator, in special cages (modified from mosquito breeding cages). Temperature, relative

humidity and light:dark cycles were controlled. These cages also served as transport devices.

Infestation was done on an infestation table. This special table was fabricated in accordance with the contractor's recommendations by the USAMRIID metal shop (Mr. Don Smith supervised this task). (Figure 1). Infestations were done under gauze cloth to contain the ticks. The isolation room was also cleared of other tables or furniture with drawers, and the floors, ceiling, drains, and door frames were treated with sticky oils or tape to prevent escape of loose ticks, if any. Animals were tranquilized to minimize violent thrashing or other behavior that might disperse the ticks. A large reflector, made of metal, was installed on the back of the table to reflect ticks thrown from the host that might fall unnoticed behind the table.

Recordkeeping was planned and discussed with the colony supervisor; initially, Mr. John Kondig, was designated for this task. A complex record form was designed that allowed the colony supervisor to record the status of all life stages, their use and ultimate fate, for each species. This form was later simplified. Computer hardware and appropriate software was acquired to support this mission.

Training in tick rearing procedures and recordkeeping methods was regarded as an essential element in the development of these tick colonies and their safe management. The contractor proposed his facilities at Old Dominion University for 3-4 days of training and instruction for designated USAMRIID personnel.

Literature needed to provide the data base for the research was acquired and a reference list constructed in DBASE. The necessary hardware and software noted above made it possible to generate a massive data base

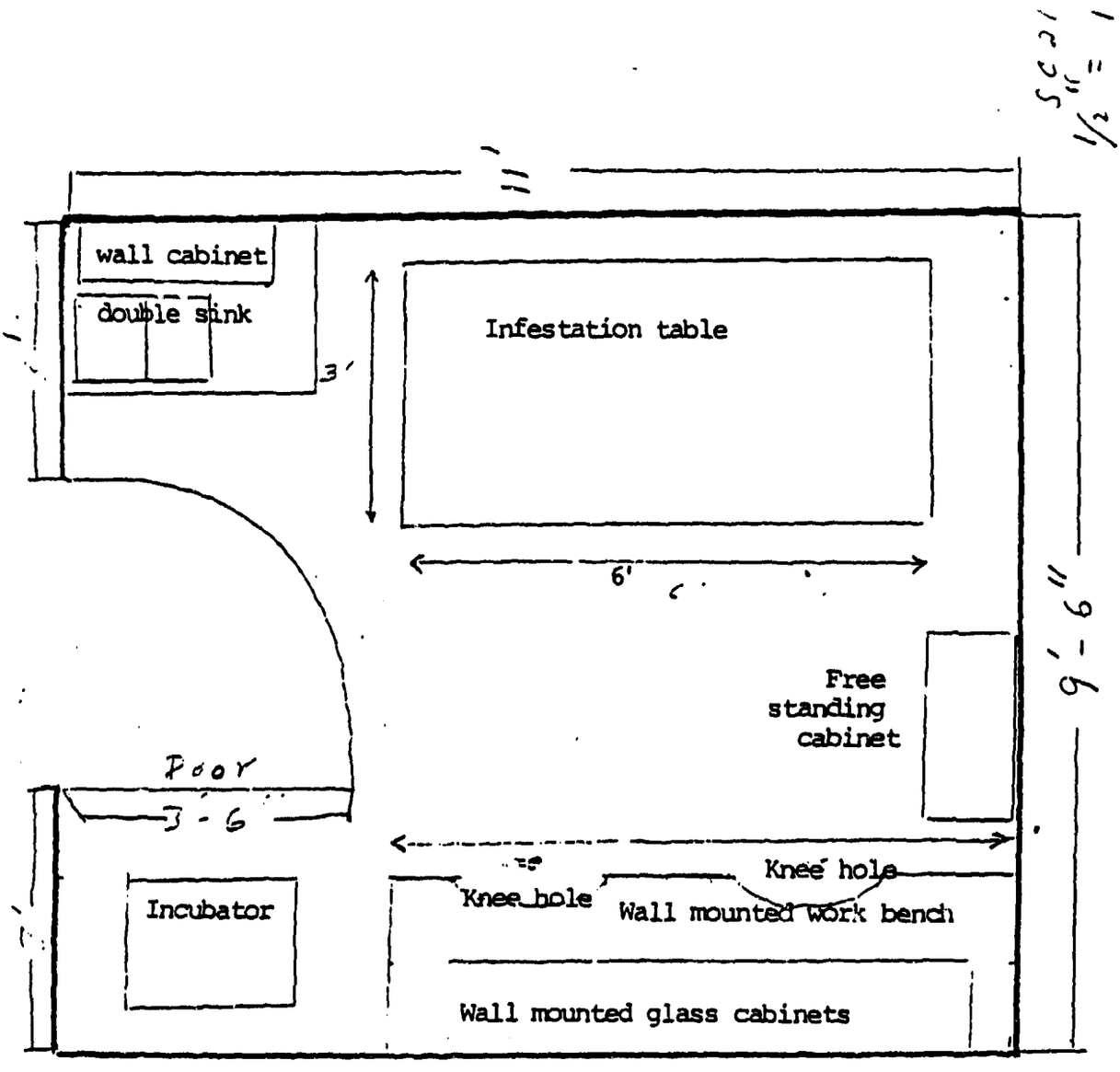

Fig. 1. Diagrammatic sketch illustrating the design of the isolation room used for feeding exotic (African) ticks on laboratory animals.

and provide retrieval services on any of a wide variety of key fields (e.g. author, journal, etc.). Floppy disks containing this library were furnished to USARAMIID personnel periodically as the library was developed.

V. RESULTS

1. Facilities and Equipment

A plan for colonization and containment of the tick, Hyalomma marginatum rufipes, was prepared and presented to Dr. Charles Bailey, Lt. Colonel, U.S. Army, and other personnel of the Department of Arboviral Entomology, USAMRIID, on April 15, 1986. Included in the meeting were personnel from other departments concerned with fabrication of special equipment for the project. A room in the USAMRIID insectary area was designated for this research, and modifications designed to meet work specifications were designed. Mr. John Konig was assigned as manager of the tick colonization program and the contact for consultations with Dr. Sonenshine, project consultant, and Principal Investigator. The design of this room was the result of meetings and discussions between Dr. Sonenshine and USARIID personnel. The room is solid wall construction, pointed, and with a monolithic floor containing a floor drain (covered). The door frame, electrical outlets, and heating ducts were treated with a sticky oil barrier to trap any ticks that might escape during specimen handling or feeding on animals. A work bench, without drawers, but with knee holes, provided the working area where personnel could handle tick specimens. A wall mounted glass cabinet provided a place for storage of only the most essential supplies, well above the work area, and, therefore, unlikely to become contaminated in the event that ticks escaped. A small incubator, free standing, was provided to house tick specimens on a temporary basis (the main incubator was in the main

insectary, the adjoining laboratory). The free-standing cabinet was available to contain bulky supplies that could not readily fit into the wall mounted cabinets. A double sink with a small drain board provided for clean up of vials, other infestation materials and for the technicians to wash after their work. Waste baskets, trash barrels, or containers for dead animals were not housed in the infestation room but could be moved in and out as needed.

A portable containment tray, Figure 2, was also designed to provide a means for storing exotic ticks during their non-feeding stages. The tray was designed to fit in an incubator, and to be removed as a unit when needed for transport to another location. The subdivisions within the tray provided for an orderly arrangement of specimen vials, so that the vials could be entered by row number and this arrangement recorded in a log book. Subsequently, USAMRIID personnel decided to modify existing mosquito breeding cages for this purpose, and these "portable containment trays" were never fabricated.

A cart for safe transport of exotic tick specimens was also designed. (See Fig. 3). This was to be fabricated at USAMRIID, using a commercially available metal or fiberglass cart as a base. The top of the cart was to contain the sides and a locking cover so that the containment trays could be installed, or, if needed, loose specimen vials could be transported safely. This modification was constructed under the supervision of Mr. Don Smith.

An infestation table was also designed and fabricated in the USAMRIID shop facility under the supervision of Mr. Don Smith. Figure 4 illustrates this table. The purpose of this table was to allow for infestation of animals by ticks so that escape of loose specimens was minimized or impossible. To accomplish this goal, a reflecting wall was build arourd the sides and

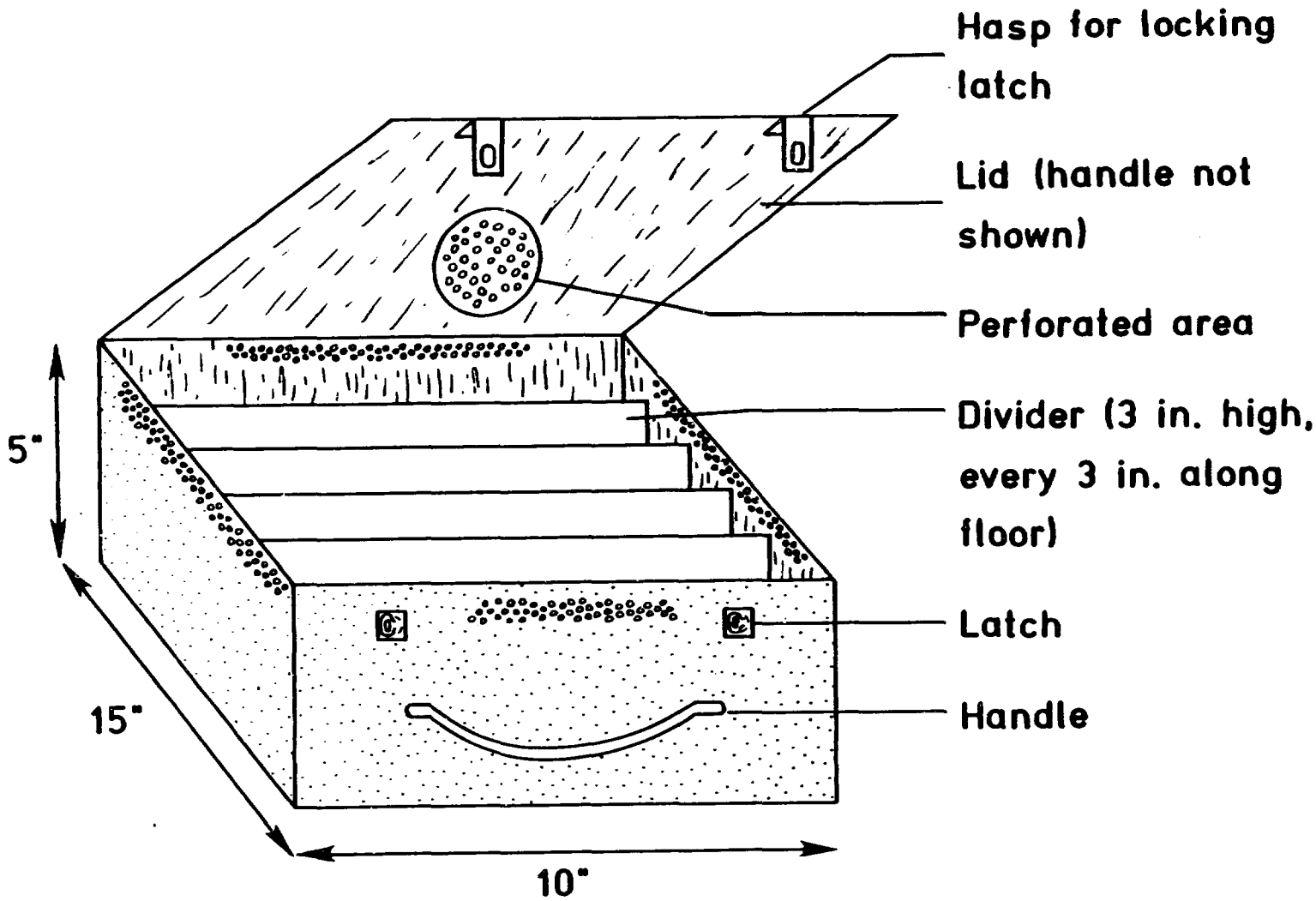

Fig. 2. Diagram of a portable containment tray to hold exotic ticks during non-feeding stages. The tray can be locked. Numerous holes and perforations provide for air exchange. The interior of the tray is divided into rows for orderly arrangement of specimen vials.

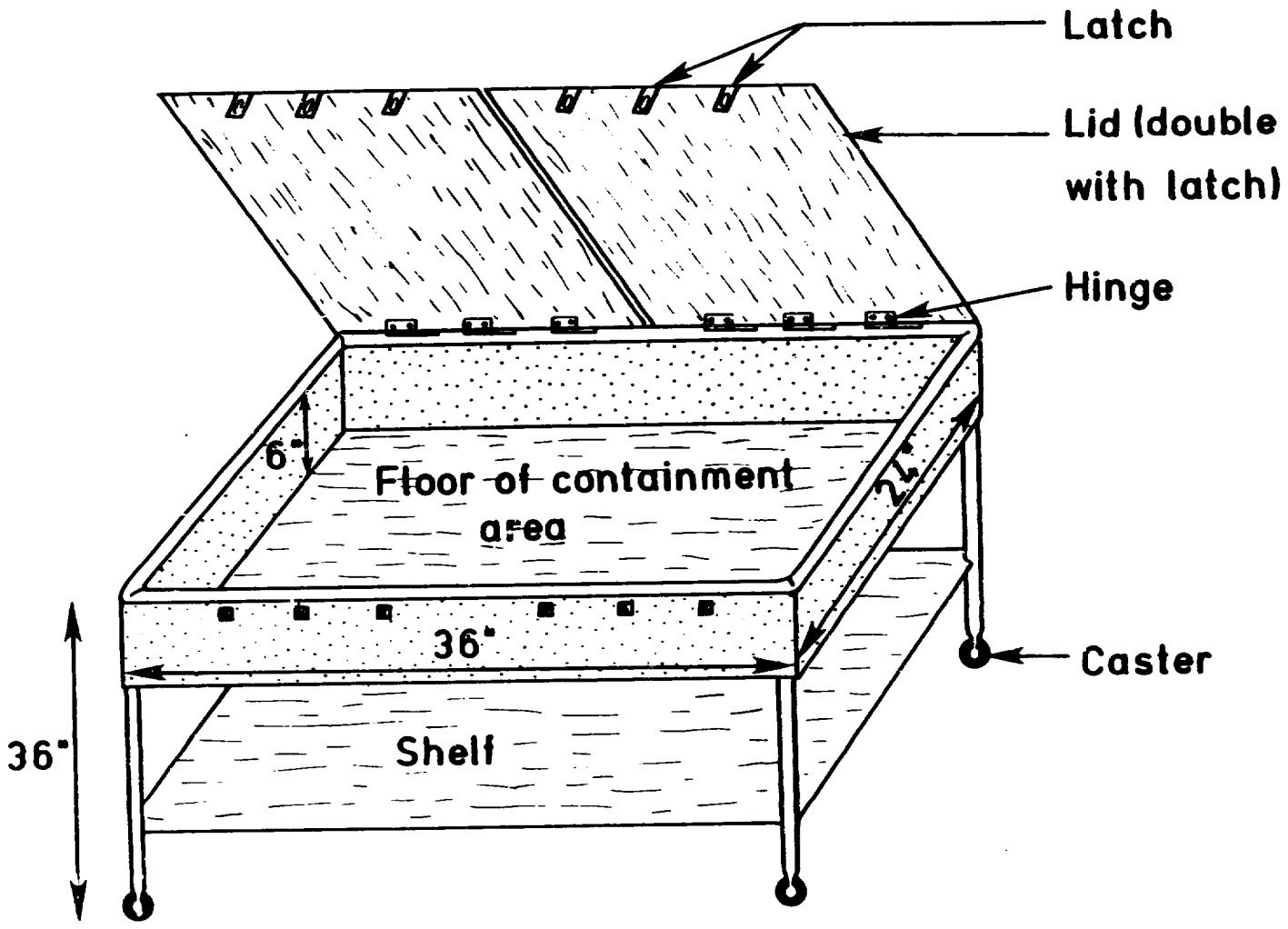

Fig. 3. Transport cart, with locking cover, for safe transport of exotic ticks between rooms.

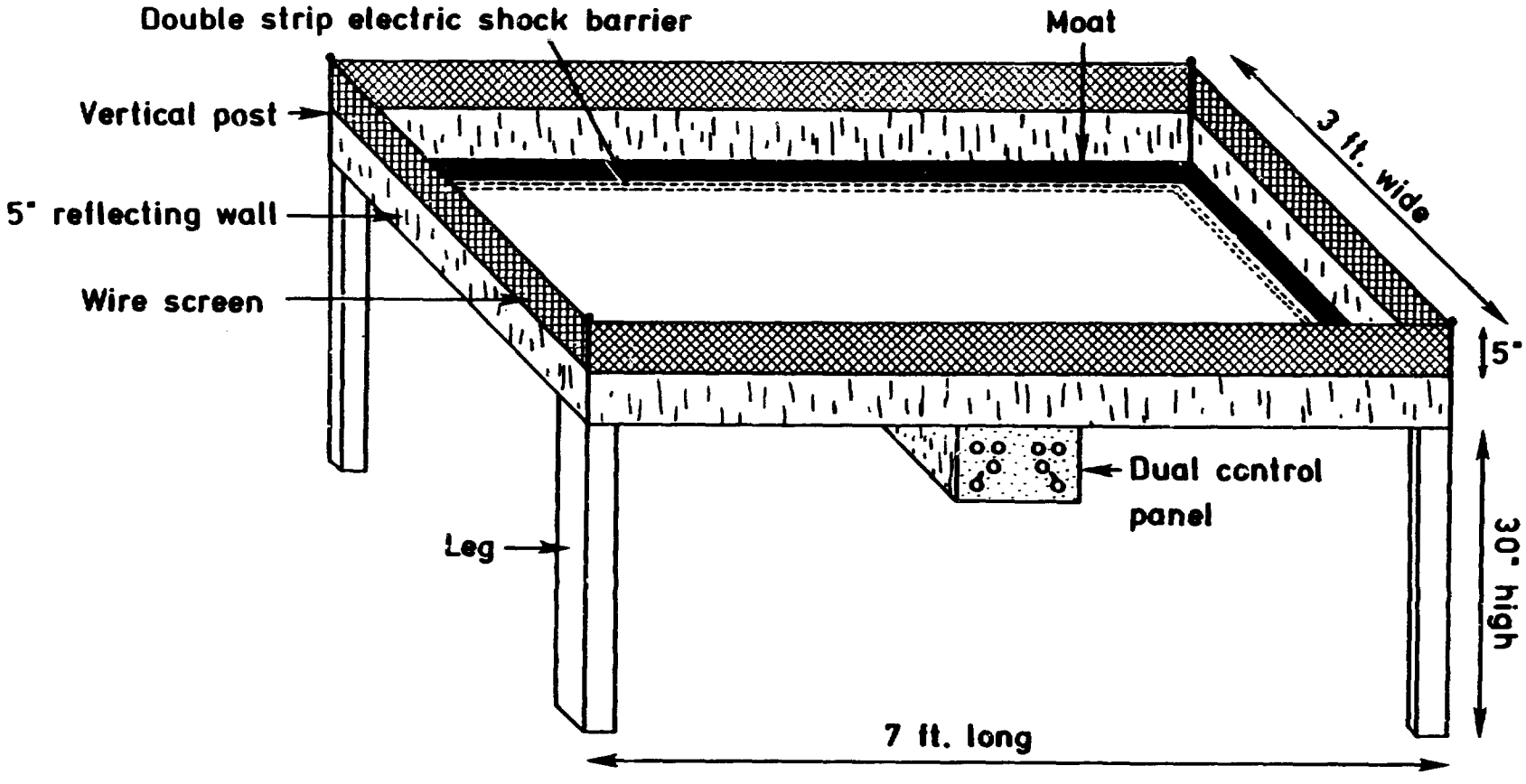

Fig. 4. Moat table with electric shock barrier, moat containing pesticide solution, and metal sides to repel ticks escaping from host animals during infestations with exotic ticks. Animals to be infected were placed in cages in the center of the table.

back of the table; the wall around the front was eliminated (shown in the figure, but eliminated in the design). A solid metal barrier was eventually substituted for the wire screen shown in the figure. Next, a double strip electric shock barrier was installed along the inner margins of the table, controlled by a control panel on the front of the table (to regulate the intensity of the current) and a liquid filled moat surrounded the entire table just inside the reflecting wall. Oil containing a non-toxic pesticide (e.g. Permethrin) could be used to kill any ticks that entered the moat. These barriers were expected to (1) deter ticks that escaped that host or, inadvertently, from the technicians, during feeding (electric shock barrier); (2) kill ticks that crossed the electric shock barrier, or were caught by the reflecting wall (moat); and (3) prevent escape of ticks thrown from the host by violent twitching, jerking or other violent movements of the animal host (metal reflecting barrier).

Incubators (not figured in this report) in the main insectary room adjoining the isolation/tick infestation room were made available to house the tick colonies during their non-parasitic stages.

2. Tick Colonies

Four species of exotic ticks were brought back from East Africa as a result of collecting trips made by USAMRIID personnel. These include Boophilus decoloratus, Hyalomma plumbeum, Rhipicephalus evertsi evertsi and Amblyomma variegatum. Engorged female specimens of each species were included in the collections, and these laid eggs. Thus, progeny of each of the 4 species are now available for initiating colonies. In addition, at the request of John Konig, I sent ca 100 adults, mixed males and females, of the American dog tick, Dermacentor variabilis, from my laboratory colony.

These ticks were to be used for initiating a colony of this species and to serve as the vehicle to practice containment procedures. Additional specimens (ca 100 adults) were provided during the visit by Captain Logan and Mr. Kondig to Old Dominion University (please see below, 3. Training).

Although the training and testing procedures with the native tick, D. variabilis, were to precede the colonization of the African ticks, travel opportunities dictated the need to acquire the latter. Consequently, testing with D. variabilis was initiated and this work was done before actual feeding of the larval stages of the African ticks was allowed. Tests with D. variabilis demonstrated the efficiency of the containment procedures and provided direct "hands on" experience for the USAMRIID personnel engaged in the work.

3. Training

During the week of August 25-28, 1986, John Kondig and Dr. Thomas Logan visited my research laboratory at ODU for training and consultations. Training emphasized breeding techniques, containment procedures, and computerized recordkeeping. Breeding techniques included hands-on experience in infesting rabbits with either American dog ticks, D. variabilis, or Rocky Mountain wood ticks, D. andersoni, and the African tick, Hyalomma dromedarii (camel tick) on rabbits and on rats. Both Mr. Kondig and Dr. Logan prepared the animals for infestation and carried out infestations themselves, thereby gaining direct experience in the techniques we use in my laboratory. To illustrate computerized recordkeeping, a colony notebook was prepared for Amblyomma maculatum, one of the species we have in colony, and one which has the smallest numbers of containers. Prior to the visit of Mr. Kondig and Dr. Logan, I numbered all of the vials of ticks, segregated them

by age and sex, and recorded the data on record forms in the colony logbook. Next, I created a file in DBASE 3 Plus, the computer software used for this purpose. I entered records of several of the vials to illustrate the principals involved in the records transfer techniques. When Mr. Kondig and Dr. Logan arrived, I explained the procedures, and asked them to create a similar file to gain experience in these DBASE procedures. Having set up the file, I asked them to enter records for this species, one record for each vial. The specific fields, e.g., vial number, date of feeding, etc., were reviewed and a discrete number of fields were established to simplify recordkeeping. Finally, the records were entered, creating a file with a complete set of records for all of the vials containing specimens of this species. When completed, I illustrated retrieval techniques. Records were retrieved by date of entry, life stage, or other specific fields that were present in the data base. In this manner, we were able to determine all of the vials for any given life stage, determine how many specimens of a given life stage were available for study, determine the status of specimens (when last fed, when molted, etc.), or other needed information. Finally, I illustrated the report creation procedures. Formal reports were generated giving information on the specific fields where needed.

A more detailed description of this training is contained in Appendix A, "Trip Report," by Captain Logan and Mr. Kondig, dated September 10, 1986.

4. Implementation of the Tick Feeding and Containment Procedures

On December 16, 1986, I travelled to USAMRIID and visited the personnel of the Disease Assessment Division to consult on the progress of the colonization of the four African ticks and their containment. I met with Mr. J. Kondig, Captain T. Logan, Mr. J. Moulton, and Colonel C. Bailey.

Construction of the tick breeding tables had been completed, with one such table located in the non-infected tick colony breeding room, or isolation room, adjoining the insectary, while the other was installed in the "hot suite," a P-3 facility for work with Crimean-Congo Hemorrhagic Fever and other highly infectious organisms. During my visit, I also observed tick feeding on a guinea pig. Specimens of Amblyomma variegatum were confined using nylon cloth glued to the shaved back of the animal (i.e., "sleeve" technique). This technique minimizes escape of larvae or nymphs. The infested animal was held on the tick breeding table in the tick colony room.

During my visit, I had an opportunity to inspect the containment procedures used in the tick colony room. The only furniture in the room was the breeding table, a small cabinet and a sink. Sticky tape covered with heavy oil ("Tac Trap") was used liberally around the walls, around the floor drain, around the vent in the ceiling, electric outlets, and even under the door (blocking the door sill). The air vent and floor drain were covered with fine mesh metal screen. Technicians handling ticks or tick infested animals in the room wore special lab coats which were removed and placed in a hamper in the room for subsequent decontamination. This is now routine procedure.

Transport of the ticks from the incubator (see below) to the tick colony room is done with special cages adapted from mosquito rearing cages. This is used instead of the portable containment tray included with the original proposal and appears to work just as well. The ticks are held in the incubator in the outer laboratory, i.e., the same laboratory where mosquito studies are being done and other technicians, working on unrelated projects, also perform their duties. Nevertheless, the fact that the ticks are held in the plastic containers described above insures that the ticks are secured and protected against risk of spread elsewhere into the

incubator. I suggested that some portion of the incubator be designated for the tick colonies, and that this part of the incubator be further subdivided for the containers used for each life stage. It would be also be desirable to concentrate the species intended for immediate study in one incubator, and retain the other species in a separate incubator. This arrangement would avoid overcrowding, and minimize the risk of confusing specimens of different species, resulting in mixed colonies.

5. Recordkeeping Procedures

A computerized tick colony data base record system was created during the first 6 months of the project, using the software package "DBASE3 Plus" by Ashton-Tate. This was done as described in the original project proposal. The record forms were intended for managing a colony of a single species, e.g., Hyalomma marginatum. Provisions were made to track the fate of each life stage, number the vials containing specimens, report the ultimate fate of the tick specimens, note their location, and generate colony status reports. These procedures were considered important to avoid inadvertent loss of tick colony material or confusion regarding specimen location. Training in the recordkeeping procedures was included in the visit by Captain Logan and Mr. Kondig. Subsequently, when it was decided to colonize four species, the complexity of the record forms became a paramount consideration. Mr. James Moulton, of the Disease Assessment Division, was assigned as tick colony manager, replacing Mr. Kondig, and Mr. Moulton produced a more simplified "Tick Colony Log," incorporating many of the items in my original record forms. Both the proposal forms and Mr. Moulton's revised version are shown in Appendix B. The revised form meets the major needs for recordkeeping.

Records are now being maintained by computer as well as in handwritten logs. Copies of suggested data base fields for tick colony records were furnished and training of Department of Arbovirology personnel in development of computerized recordkeeping was also done. Transfer of computerized records via modem between the Department of Arbovirology and ODU was discussed. No suitable telephone is available in the insectary. Although modem to modem transfer could be accomplished by carrying the floppy disk containing the records to a suitably equipped computer system elsewhere in the facility, no plans are being made to do so at this time. The laboratory technician, Mr. Moulton, will forward records to the Principal Investigator for inspection if further assistance in recordkeeping is desired.

6. Computer Based Tick Literature File

A massive file containing more than 700 citations relevant to Crimean-Congo Hemorrhagic Fever and its vector ticks was created. I arranged for an ODU computer programmer to write a special program to report the records in citation format, i.e., in the bibliographic style. Using the "DO" command, this program (SPITANA) supercedes the DBASE reporting procedures, and cites the selected records in bibliographic format. Thus, the advantages of DBASE 3 PLUS, namely, record retrieval by specific field, e.g., author, date, keywords, etc., can be used to maximum advantage. Having the massive literature file available on a microcomputer enables us to call up information that is needed for colony maintenance, infestation techniques, viral transmission techniques, host sensitivity to ticks and/or viral pathogens, and so on. We have already demonstrated the usefulness of this capability in our ability to answer Mr. Kondig's request for information on the feasibility of feeding Hyalomma spp on guinea pigs. A copy of this file is being

furnished, both on a floppy disk and a hard copy. We will continue to add to these records and furnish them to USAMRIID as a continuing service and good will gesture.

7. Other

Although not included in the original project, special assistance was requested and provided regarding tick anatomy, techniques for identifying and excising tick body organs, and for culturing/maintaining tick cells and organs. In addition, we provided supplies of "Yunker-Meibos" growth medium made at ODU (see Appendix C, letter from Dr. Paul Homsher). We routinely maintain tick cells in culture in facility at ODU, using the Yunker-Meibos cell culture medium. This medium has proven to be excellent for D. variabilis cell culture, and has been reported to be suitable for culture of cells from other species also (Yunker, C. E., J. Cory, and H. Meibos 1984. Tick tissue and cell culture: applications to research in medical and veterinary acarology and vector borne disease, p. 1082-88. In: D. A. Griffiths and C. E. Bowman, eds., Acarology VI Vol. 2, Ellis Horwood, Ltd., Chichester). We will continue to collaborate on a good will basis as is common among scientists sharing common interests.

VI. DISCUSSION AND CONCLUSIONS

This project furnished expertise and training that made it possible to colonize and safely contain four species of exotic African ticks needed for studies on the transmission of Crimean-Congo Hemorrhagic Fever. The project furnished designs for equipment, concepts for electronic recordkeeping procedures using computer software, a literature library of more than 700 pertinent records, and specialized training. The equipment, facilities, procedures and experience gained may serve as a useful model for other

laboratories contemplating work with vector arthropods and transmission of highly contagious microbes.

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APPENDIX A

Trip report by Captain Thomas Logan and Mr. John Kondig, September 10, 1986, describing the results of their visit to Old Dominion University for training in the safe handling of exotic ticks.

Best available Copy

14 Jan 88

OSITION FORM

. this form, see AR 340-15; the proponent agency is TAGO.

ENCE OR OFFICE SYMBOL	SUBJECT
SGRD-UID-A	Trip Report

XX THRU C, Dept Arbo Ent
C, Disease Assessment Div
Security Manager (C, RMO)
Deputy for Research Support
Deputy for Product Development
Deputy for Research

FROM CPT Logan/Mr. Kondig

DATE 10 Sep 86

CMT 1
dd/7244

TO Commander, USAMRIID

1. Activity visited: Old Dominion University, Norfolk, Virginia, was visited from 25 to 28 August 1986. Travel was performed under Travel Order MRI 8-9 and MRI 8-10 dated 6 August 1986.

2. Purpose: To study procedures for handling exotic species of vector ticks at Old Dominion University.

3. Persons contacted: Daniel E. Sonenshine, Ph.D., Associate Vice President for Research, Old Dominion University; Dr. Paul Homsher, Assistant Dean, Old Dominion University, Norfolk, VA; Mr. DeMar Taylor, graduate student; Mr. Gordon Hamilton, graduate student; Mr. Martin Schreifer, graduate student.

4. Findings: The procedures for handling exotic species of vector ticks, developed by Dr. Sonenshine, has been successfully applied to the establishment and maintenance of Hyalomma dromedarii. Dr. Sonenshine's procedures provide for an independent area that is used to handle all aspects of rearing and maintaining this species. The techniques used to maintain this colony are similar to those used to maintain established colonies of indigenous ticks.

Mr. Martin Schreifer, who maintains the colony of H. dromedarii, explained the procedures used to assure containment of the colony to a specific area; time was then spent within the containment area observing the techniques used in handling the ticks. Mr. Schreifer discussed the problems involved in developing techniques for handling exotic ticks from the point of view of being able to account for all ticks, in all life stages.

Mr. DeMar Taylor explained the various techniques used to blood feed ticks at their various life stages. The most useful technique, from the point of view of safety and containment, is the use of a capsule attached to the side of the host animal. Mr. Taylor demonstrated the technique of taping a capsule to the side of a rabbit and discussed the problems that might be encountered with the procedure. Experience was gained in the use of this and other techniques by personally infesting ticks on various host animals.

Important to the colonization of ticks are accurate records of the time periods between the various life stages, primarily the length of time for blood feeding and the time between blood meals. To facilitate the maintenance of records, Dr. Sonenshine developed a format for computerized data base system. Mr. Gordon Hamilton, who utilizes the system for maintaining records, explained the reasons for developing this specific format. Under the guidance of Mr. Hamilton, time was spent in learning how to enter data, how to manipulate the data in order to develop specific types of reports, and how to prepare a variety of reports. A copy of the system was brought back to USAMRIID in diskette form.

Dr. Paul Homsher discussed and demonstrated the techniques for maintaining tick cell cultures. Professor Sonenshine and Mr. Taylor demonstrated and assisted in dissection

techniques for locating and removing various organs in engorged and unfed ticks. Specimen: of Dermacentor variabilis ticks were brought back to USAMRIID for gaining technical expert in colony rearing and dissection techniques.

Dr. Sonenshine, in his discussions on rearing ticks, suggested modifications of his established procedures and techniques relative to the handling of virus infected ticks. H feels that even with the restricted conditions imposed on handling infected ticks his procedures can be modified to meet safety requirements. Dr. Sonenshine stressed the importance of accurate records in meeting safety requirements, in that they would account all ticks during the various handling procedures.

5. Summary: Some of the above techniques, with modifications, can be applied to raising maintaining several species of exotic ticks in the Insectary of the Department of Arbovir Entomology. The need for accurate record keeping is important from both the standpoint of confinement and rearing ticks. Computerizing records, based on Dr. Sonenshine's format, fulfills the need for a workable system of record keeping.

Thomas M. Logan

THOMAS M. LOGAN, Ph.D.
CPT, MS
Department of Arboviral Entomology
Disease Assessment Division

John P. Kondig

JOHN P. KONDIG
Research Chemist
Department of Arboviral Entomology
Disease Assessment Division

Best available Copy

MC 14 Jan 88

OSITION FORM

...m, see AR 340-15; the proponent agency is TAGO.

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...ORM 2400

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Thomas M. Logan

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CPT, MS
Department of Arboviral Entomology
Disease Assessment Division

John P. Kondig

JOHN P. KONDIG
Research Chemist
Department of Arboviral Entomology
Disease Assessment Division

APPENDIX B

Samples of computer-based recordkeeping forms.

"Designed by personnel of the Disease Assessment Division,
USAMRIID, Fort Detrick, Frederick, MD."

TICK COLONY LOG

Species_____

1. Vial_____ 2. Gen_F___3. DO Date_____ 4. Ovip. Dates_____
5. Hatch Dates_____ 6. Use_____7. Date Infested_____
8. Host_____ 9. No. Infested_____

Vial	DO Date	# in Vial	Molt Date	# Used For	Date used	Host

Cart	DO Date	# in Cart	Molt Date	# Used For	Date Used	Host	Comments/Final Disposition

"Form designed by Dr. Sonenshine, ODU"

Tick Colony Form No. 1

TICK COLONY STATUS AND USE LOG

Free living stages only

1. Species:_____ 2. Life stage:_____

3. Vial No. _____ 4. Feeding status (fed/unfed):_____

5. No. in vial (see expl.) _____ _____

6. Location:

   a) Incubator No. _____; b) tray No. _____

7. Date of first record: _____ 8. Molted to: _____

9. Date molted:_____ 10. No. molted: _____

11. No. died without molting:_____ 12. Used for (see expl.):_____

13. No. remaining:_____

14. Date of next record: _____

15. Status (see expl.): _____ 16. Used for: _____

17. No. remaining:_____

18. Date of next record: _____

19. Status: _____ 20. Used for: _____

21. No. remaining:_____

22. Date of next record: _____ 23. Status:_____

24. Used for:_____ 25. No. remaining:_____

26. Final disposition (see expl.):_____

_____

Explanations:

"Form designed by Dr. Sonenshine, ODU"

(1) Items 1 - 4: self explanatory.

(2) Item 5: estimate number of unfed larvae from data on egg mass weight and visual approximation of the percentage of eggs that hatched.

(3) Items 6 - 11: self explanatory.

(4) Item 12: Describe purpose of use, e.g., used to infest rabbit, inoculated with viruses, etc.

(5) Items 12-14: self explanatory.

(6) Item 15: Describe condition of specimens, e.g., vigorous, torpid, dessicated, etc.

(7) Items 16-25: self-explanatory.

(6) Item 26: Describe what was done with the vial and remaining specimens, e.g., ticks destroyed, vial discarded.

"Form designed by Dr. Sonenshine, ODU"

Tick Colony Form No. 2

TICK COLONY STATUS AND USE LOG

Tick Feeding Activity

1. Species:_____ 2. Vial No. _____

3. Life stage: _____ 4. Host: _____

5. Date infested: _____ 6. No. attached: _____

7. 1 st. date ticks recovered: _____ 8. No. recovered: _____

9. 2 nd " " " _____ 10. No. recovered: _____

11. 3 rd " " " _____ 12. No. recovered: _____

13. 4 th " " " _____ 14. No. recovered: _____

15. 5 th " " " _____ 16. No. recovered: _____

17. Final date all recovered: _____ 18. Total No. recovered:_____

19. Date of next record: _____ 20. Status:_____

21. Date of 1 st. molt:_____ 22. No. molted:_____ 23. No. died:_____

24. " " 2 nd " _____ 25. No. " _____ 26. No. " _____

27. " " 3 rd. " _____ 28. No. " _____ 29. No. " _____

30. Date all molted:_____ 31. Totl molted_____ 32. Totl died_____

"Form designed by Dr. Sonenshine, ODU"

Tick Colony Form No. 3

OVIPOSITION RECORD

1. Species: _____. 2. Vial No.:_____

3. No. engorged females: _____ 4. Date replete:_____

5. Weight all females in vial: _____

6. Location:

   (a) Incubator No.:_____

   (b) Tray No.: _____

7. Date ovip. began:_____ 8. Wgt. eggs/end ovip.:_____

9. Date 1 st hatch:_____ 10. Date hatch complete:_____

11. Est. hatching success (see expl.): _____

12. Date last record:_____ 13. Status:_____

14. Used for:_____

15. No. remaining:_____

16. Final disposition: _____

Explanations:

(1) Items 1 - 10: self explanatory.

(2) Item 11: Estimate the percentage of eggs that hatched.

(3) Items 12 - 15: self explanatory; see explanation for final disposition given with form No. 1.

APPENDIX C

Letter from Dr. Paul Homsher concerning provision of media to culture tick cells.

OLD DOMINION UNIVERSITY

Office of the Dean
School of Sciences and Health Professions
Norfolk, Virginia 23508-8540
Telephone (804) 440-3274

September 29, 1986

Mr. John Condig
Arboviral Entomology
Disease Assessment Division
USAMRIID
Fort Detrick, Maryland 21701-5011

Dear John:

I understand that you can use the cells now, so Lynn Ellis is making up two flasks for you and should be sending them on early next week. She will also include a small bottle of our media to keep you going until you can make your own. When you receive the flasks, pour off the media covering the cells and add fresh (I have spun the pour off to rid it of any floating cells and used it successfully for conditioning flasks and, in an Emergency, feeding the cells). If the cells have sloughed off for any reason, I would spin the cells from the media (900-1000 RPM) and reinoculate the flasks, adding fresh medium. Do not hesitate to call me if I can help.

It was nice meeting you and I wish you success in using the cells.

Sincerely,

Paul J. Homsher, Ph.D.
Associate Dean

PJH:lsd

pc: Lynn Ellis
Dan Sonenshine

APPENDIX D

Computer-Based Tick Literature File

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