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RECOMMENDATIONS ON EXPERIMENTAL USE OF
AEDES AEGYPTI

With the development of the Aedes aegypti eradication programme in the United States of America it became apparent that special attention would have to be given to the laboratory colonies maintained in many institutions throughout the United States and territories. Because the colonies present a potential threat to eradication, concern was expressed from many quarters that continuation of such colonies would permit reinfestation of areas from which A. aegypti had been eradicated.

A resolution was passed by the Entomological Society of America (ESA) on 5 December 1963, calling for discussions by a committee representing the ESA and the United States Public Health Service (USPHS) to devise plans and proposals for the safe maintenance of laboratory research colonies of A. aegypti compatible with nation-wide eradication of this mosquito. This resolution was endorsed by the American Mosquito Control Association on 4 March 1964, and by the Armed Forces Epidemiological Board in May 1964.

Accordingly, the President of ESA appointed a committee on experimental use of A. aegypti which represented the various professional and government agencies with an interest in the problem. Professor Don W. Micks of the University of Texas is chairman of this committee.

The United States Public Health Service, after careful consideration of the reasons both for and against laboratory colonies, reached a decision to permit the retention of laboratory colonies at this time provided that they are maintained under proper controls.

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A working conference on A. aegypti laboratory colonies was held in Atlanta on 21 May 1965, to allow discussion of the many aspects and problems of colony maintenance. As a result of this and numerous other meetings, and in the light of the USPHS decision, the ESA Committee on Laboratory Use of A. aegypti under the chairmanship of Professor Don W. Micks, released in October 1965 a "summary of recommendations" for laboratory colonies.

This document represents the culmination of considerable effort to devise a mutually agreeable plan whereby A. aegypti colonies could be maintained without jeopardizing the eradication programme.

Much information on this problem will be found in the text of six articles presented on 5 November 1965 at a symposium on the eradication of Aedes aegypti in the United States of America (Amer. J. trop. Med. Hyg. 1965, 14, 885-912).

The ESA Committee requested that those who at present maintain colonies or anticipate working with A. aegypti carefully study this material with the view of adopting and adapting these recommendations to individual laboratories.

The recommendations cover the following main points:

- (1) non-essential colonies of A. aegypti are to be eliminated;
- (2) importation of A. aegypti from abroad is to be discouraged.
Only when strong justification exists may certain strains be imported;
- (3) a permit system for interstate shipment within the United States is to be instituted;
- (4) laboratories maintaining colonies must be certified to be escape-proof;
- (5) surveillance is to be maintained around certified laboratories to ensure against accidental escape.

The United States Public Health Service is to develop the permit system, make provision for laboratory certification, and carry out surveillance procedures as soon as feasible. Provision will be made for a review panel, comprised of experts both in and out of government, to hear appeals on any decisions made by USPHS in regard to laboratory colonies.¹

The World Health Organization, realizing the importance and practical value of these guidelines to all entomological laboratories working with any mosquito colonies, feels that this document should be more widely known. This aim can be achieved through releasing it in the WHO/Vector Control and WHO/Mal cyclostyled documents.

It is obvious that the recommendations as set forth in the ESA document apply specifically to colonies of Aedes aegypti and should not be understood as referring to any mosquito colonies irrespective of the species involved. This would impose unnecessary limitations on the use of other mosquito colonies and may interfere with the progress of entomological research. Nevertheless, the security measures voluntarily accepted with regard to Aedes aegypti may be regarded as a pattern for adoption of similar measures in laboratories working with anopheline or culicine mosquitos which are known as vectors of human disease, wherever there should be any possible conflict between public health problems and research needs.

Our thanks go to Professor Don W. Micks of the University of Texas, Galveston, Texas, for permission to reproduce the ESA original document and the background information.

¹ "The Transmitter" No. 5, December 1965 (Cyclostyled document issued by the Aedes aegypti Eradication Branch, Communicable Disease Center, Atlanta, Georgia, United States of America

RECOMMANDATION CONCERNANT L'EXPERIMENTATION AVEC Aedes aegypti

A mesure que le programme d'éradication d'Aedes aegypti progressait aux Etats-Unis d'Amérique, il est apparu que l'on devait se préoccuper tout spécialement des colonies de moustiques élevées dans les laboratoires de nombreuses institutions des Etats-Unis et de leurs territoires. Ces colonies pouvant constituer une menace pour l'éradication, on a exprimé de divers côtés la crainte qu'elles ne favorisent la réinfestation de secteurs déjà débarrassés de la présence de ce moustique.

Le 5 décembre 1963, l'Entomological Society of America (ESA) a formulé une résolution demandant qu'une commission représentant l'ESA et le Service de Santé publique des Etats-Unis (USPHS) soit chargée d'élaborer des plans et des propositions pour que l'élevage de colonies d'A. aegypti dans les laboratoires de recherche se fasse dans des conditions de sécurité compatibles avec le programme national d'éradication de ce vecteur. Cette résolution a été entérinée par l'American Mosquito Control Association le 4 mars 1964 et par l'Armed Forces Epidemiological Board en mai 1964.

En conséquence, le Président de l'ESA a nommé une "Commission pour l'utilisation expérimentale d'A. aegypti" (Committee on Experimental Use of A. aegypti) où sont représentés les divers organismes professionnels et gouvernementaux intéressés. Le Dr Don W. Micks, de l'Université du Texas, en a été nommé Président.

Après avoir soigneusement examiné les arguments en faveur ou contre le maintien de ces colonies, le Service de Santé publique des Etats-Unis a décidé d'autoriser, pour le moment, l'élevage de colonies d'A. aegypti au laboratoire, à condition que toutes les précautions soient prises pour en assurer la surveillance.

Une "Conférence de travail sur l'élevage d'A. aegypti au laboratoire" s'est réunie à Atlanta le 21 mai 1965 pour étudier les multiples aspects du problème. A la suite de ses travaux et de ceux de nombreuses autres réunions, et compte tenu de la décision prise par l'USPHS, la Commission de l'ESA pour l'utilisation expérimentale d'A. aegypti a publié en octobre 1965 un "Abrégé des recommandations" relatives aux colonies de laboratoire.

Ce document représente donc l'aboutissement des efforts très importants par lesquels on a voulu concilier les deux points de vue opposés et maintenir l'élevage d'A. aegypti au laboratoire sans risquer de nuire au programme d'éradication.

On trouvera une abondante documentation sur cette question dans le texte de six communications présentées le 5 novembre 1965 à un symposium sur l'éradication d'Aedes aegypti aux Etats-Unis d'Amérique (Amer. Journ. Trop. Med. Hyg., 1965, 14, 885-912).

La Commission de l'ESA a demandé à tous les laboratoires qui élèvent des colonies d'A. aegypti ou prévoient d'en utiliser pour leurs travaux, d'étudier soigneusement ce document afin d'adopter les recommandations qu'il contient en les adaptant à leurs besoins particuliers.

Ces recommandations portent principalement sur les points suivants :

- 1) Il faut supprimer les colonies d'A. aegypti qui ne sont pas indispensables.
- 2) L'importation d'A. aegypti est à décconseiller, sauf raisons majeures justifiant l'emploi de certaines souches en provenance de l'étranger.
- 3) Il faut créer un système d'autorisation pour le transport de moustiques d'un Etat à l'autre à l'intérieur des Etats-Unis.
- 4) Les laboratoires qui élèvent des colonies d'A. aegypti doivent se faire délivrer un certificat attestant qu'ils sont installés de telle façon que les moustiques ne peuvent s'en échapper.
- 5) Les alentours de ces laboratoires doivent faire l'objet d'une surveillance destinée à déceler toute fuite accidentelle des vecteurs.

L'USPHS instituera le plus rapidement possible le régime d'autorisations et d'attestations et le dispositif de surveillance. Un comité composé d'experts appartenant à l'administration et de personnalités non officielles sera constitué pour examiner les appels contre les décisions prises par l'USPHS en ce qui concerne les colonies de laboratoire.¹

¹ "The Transmitter" No 5, décembre 1965 (document ronéotypé, publié par : Aedes aegypti Eradication Branch, Communicable Disease Center, Atlanta, Géorgie, Etats-Unis d'Amérique).

L'Organisation mondiale de la Santé, consciente de l'importance et de l'intérêt pratique de ces directives pour tous les laboratoires d'entomologie qui utilisent des colonies de moustiques pour leurs travaux, estime que ce document devrait faire l'objet d'une plus large diffusion et propose, à cet effet, de le publier dans la série de documents ronéotypés WHO/Vector Control et WHO/Mal.

Il est évident que les recommandations contenues dans le document de l'ESA s'appliquent spécifiquement aux colonies d'Aedes aegypti et non aux moustiques de toutes espèces. En généralisant, la portée reviendrait à restreindre inutilement l'emploi d'autres colonies de moustiques et pourrait faire obstacle aux progrès de la recherche entomologique. Toutefois, les mesures de sécurité volontairement acceptées pour Aedes aegypti pourraient servir de modèles à des laboratoires qui élèvent des anophèles ou des Culex vecteurs de maladies humaines chaque fois que les besoins de la recherche risqueraient d'entrer en conflit avec la protection de la santé publique.

Nous adressons nos remerciements au Professeur Don W. Micks de l'Université du Texas, Galveston, Texas, qui nous a autorisés à reproduire le document original de l'ESA et la documentation de base.

SUMMARY OF RECOMMENDATIONS

Committee on Experimental Use of Aedes aegypti
Entomological Society of America, October 1965

I. Elimination of non-essential A. aegypti colonies

1. Mosquito species other than A. aegypti to be utilized for general insecticide screening, classroom demonstrations, displays, etc.
2. A. aegypti colonies not absolutely required for scientific purposes to be eliminated.

II. Importation of A. aegypti from abroad

1. Importation of exotic strains to be discouraged.
2. Importation of exotic strains when permitted should require strong justification, including scientific merit aspects, location of requesting laboratory in the United States, physical facilities and operational procedures of requesting laboratory, and source of strains to be imported. Each request for importation to be handled on an individual basis.

III. Shipment of A. aegypti in the United States

1. A permit system for shipment of living stages between laboratories to be developed, relating to certification of laboratories, identification of strains being shipped, justification of need for shipment, and location of requesting laboratory.
2. Interim procedures pending establishment of permit system to be instituted:
 - (a) indiscriminate shipment of living stages to be discontinued immediately;
 - (b) shipments to laboratories in the United States without already established colonies or without established research programme involving A. aegypti to be discouraged;
 - (c) a log of all shipments sent or received to be maintained.

IV. Certification of laboratories

1. Criteria and specifications for certification of laboratories, including measures to insure against accidental release, to be developed jointly by USPHS and ESA Committee: such criteria to include but not necessarily be limited to physical facility, operational procedures, personnel qualifications, and scope of programme.
2. In conjunction with the development of criteria and specifications, site visits to be made to various laboratories with colonies now in existence.
3. In the interim, laboratories to be encouraged to upgrade their own facilities and operational procedures to the fullest extent possible. (See Attachment I, "Recommendations for the prevention of escape of Aedes aegypti from rearing facilities.")

V. Surveillance around laboratories that maintain colonies

1. Specific procedures for surveillance around certified laboratories that maintain colonies to be developed as soon as possible.
2. Interim measures to detect presence of A. aegypti pending development of specific procedures to be instituted, including placing of oviposition containers, use of adult male traps, observation to detect escapes from colonies, etc. (See Attachment II, "Surveillance procedure for Aedes aegypti.")

VI. Implementation of recommendations

The USPHS to make the final decision regarding method of implementing permit system, laboratory certification and surveillance procedures.

VII. Appeal

In the event that an individual or an organization wishes to appeal any decision made by the USPHS regarding a laboratory colony, provision to be made for a review panel which shall make recommendations to USPHS. Such panel to be comprised of consultants who represent both governmental and non-governmental research laboratories utilizing A. aegypti colonies, as well as the Entomological Society of America, American Mosquito Control Association or other professional societies deemed appropriate by USPHS. The USPHS reserves the right to make the final decision regarding an appeal.

RECOMMENDATIONS FOR THE PREVENTION OF ESCAPE OF
AEDES AEGYPTI FROM REARING FACILITIES¹

The general principle of preventing mosquito escape is to eliminate adult dispersal from the primary source of use or of production. The greater the security maintained at the primary source, the higher the level of protection. Secondary and tertiary protective measures are of value but adherence to adequate security at the source should receive the major emphasis.

The recommendations to follow should be applicable to most small to moderate colony operations. Very large or mass-rearing operations may demand modification of detailed recommendations while adapting to the same general principle.

A. Facility requirements

1. Building: construction should be windowless, with all air exits protected by filters. In an existing building, windows in rooms containing A. aegypti should be sealed permanently or double screened.

2. Room layout:

(a) If rearing room and laboratory are not one and the same, then the rearing room should be an annex of the laboratory room and open only into it. The laboratory room should have only one entrance from it, and this should be through an ante-room, i.e. a small vestibule with entry and exit doors. The ante-room should be completely empty and have white walls. The doors of the ante-room should close automatically, and some arrangement made so that both doors cannot be opened simultaneously. The ante-room should be fitted with some protective barrier such as netting or an air curtain using recirculated and filtered air.

(b) In the laboratory and rearing rooms the walls and ceiling should be painted with a light shade of gloss enamel, preferably white. Floor should be light in colour.

¹ Attachment I (October 1965)

- (c) All drains must enter into a closed system.
- (d) Where insects are handled under hoods with air vents to outside, such vents must be screened or filtered.

B. Laboratory and rearing procedures

1. Eggs:

- (a) Store eggs in the rearing room.
- (b) Treat excess eggs after hatching with hot water or chemicals to kill the unhatched eggs before putting them into the sewerage system.

2. Larvae and pupae:

(a) Larvae:

- (i) in laboratories that do not maintain seven-day week operations, the larvae should be reared in containers with tight-fitting lids to prevent any accidental emerging adults from escaping;
- (ii) in laboratories that conduct daily rearing operations seven days a week, the use of tight-fitting lids is optional.

(b) Pupae:

- (i) pupae should be routinely collected, so that no adults emerge in the larval rearing containers;
- (ii) pupae should be in special containers, for emergence into adult cages, so that the pupal container and excess pupae can be removed from cage without adults escaping;
- (iii) unneeded larvae and pupae and discarded rearing water should be treated by heating or with chemicals, so that only dead waste materials enter the sewerage system.

3. Adults:

(a) Cages need to be escape-proof in design, without the conventional cloth sleeve. The design should provide for the prevention of escape during blood-feeding, egg collecting, sampling, introducing pupae, etc. (The host should not be introduced directly into the cage, i.e. there should be a screen barrier between the mosquitos and the host.)¹

(b) No sugar, sugar solution, or fruit should be left exposed in the rearing room.

(c) Hosts for blood feeding:

(i) animal room separate from rearing room - host animal after use should be checked for mosquitos and then placed immediately in a screen carrying case for transport to the animal room;

(ii) animals housed within rearing rooms - host animal after use should be checked for mosquitos and then placed in an animal cage which should be kept within a screened enclosure.

(d) The greater number of insects reared and the number of personnel involved, the greater the potential threat of escapes. Consequently, employees must be trained to handle the mosquitos and learn that "loose" mosquitos are not tolerated. Careless workers should be replaced with competent personnel.

(e) Do not remove any live stage of the insect from security conditions.

(f) All insects transported from one secure area to another must be housed within a solid surface container that cannot be accidentally opened.

4. Traps:

(a) An animal-baited black trap should be maintained in a dark corner of the room.

¹ Bar-Zeev, M. & Galun, R. (1960) A mosquito tight cage, Mosq. News, 20 (3), 316

McCray, E. M. Jr (1963) Escape-proof colony cage (Aedes aegypti), Mosq. News, 23 (4), 309

(b) Several oviposition-surveillance-containers should be in laboratory and rearing room(s). (See separate recommendations for escape detection.)

5. Personnel: access to rearing and laboratory facilities that use Aedes aegypti should be limited to authorized personnel who understand the need to retain all live stages under security conditions.

SURVEILLANCE PROCEDURE FOR Aedes aegypti¹

The surveillance procedures outlined below represent the current status of research now being conducted to develop methods for detecting Aedes aegypti in and around human habitations. Because of the continuous nature of these investigations, modifications or additions are anticipated. Where significant improvements occur, information on the same will be sent to all laboratories that are using the survey method described below. Since the evaluation of any technique for general use is enhanced by its application under various conditions, suggestions from all laboratories that employ this survey method would be welcome.

PROCEDURE

The purpose of surveillance is to evaluate the security of the protective measures established to prevent mosquito escape. As with the preventive escape measures, the surveillance should begin at the primary source of the mosquitos within the building and extend from there to the outside environs. Inasmuch as spot inspections can be misleading or erroneous, a system of continuous inspection² should be established. The oviposition site system currently in use at Savannah, Georgia, by the Communicable Disease Center Laboratories was developed to serve as a method for the objective detection of A. aegypti breeding in communities.

¹ Attachment II (October, 1965).

² In northern areas outside inspection would cease during winter periods.

I. Oviposition survey

A. Remove or protect from infestation all potential breeding containers on the premises. Those containers which cannot be removed or protected should be inspected routinely and thus used for survey purposes.

B. Select inspection sites within and surrounding the building in which the insects are housed.

(1) Inside building: set up sites in:

(a) rooms where insects are housed or handled;

(b) halls adjacent to outside exits;

(c) intervening areas between room and exits if advisable.

(2) Outside building: set up sites at:

(a) approximately 150-foot (45 m) intervals around building within 25 feet (8 m) of the building;

(b) adequate sampling sites up to 300 feet (90 m) from the building.

(3) The number of oviposition containers used at a site depends on the presence of the other oviposition sites, e.g. a greater number of containers are needed in a rearing room than in a colony room.

C. Selection of sites:

(1) Inside building: place oviposition jars in darkened corners or areas of the room.

(2) Outside building: place jars in bushes or shrubbery or shady areas rather than in open sunny spots.

D. Frequency of inspection: collect and examine applicator sticks twice weekly.

E. Records: maintain a record of + or - findings for all sites.

II. Preparation of oviposition attractant container

A. Materials

- (1) One-pint tapered glass jar (Atlas 723 or equivalent).
- (2) Wooden tongue depressor (6"/15 cm).
- (3) Brown blotting paper.
- (4) Two-dram homeopathic vial.
- (5) High gloss black enamel.
- (6) Masking tape (1"/2.5 cm) flexible wire 0.064" (2.5 mm) diameter.
- (7) Ethyl acetate or methyl butyrate.

B. Preparation

- (1) The cover of the jar is discarded and the jar itself coated on the outside with high gloss black enamel.
- (2) The wooden tongue depressor is used to hold a 4-3/4" (12 cm) strip of brown blotting paper on which the eggs are collected. The strip is wrapped about 1/4" (6.3 mm) from one end of the depressor to overlap itself on one broad surface. The strip is cut off flush with the side of the depressor, and stapled to it at each end.
- (3) An identification number is written on the exposed end of the depressor. The depressor is then fastened vertically against the inner side of the jar wall with masking tape.
- (4) The vial is suspended about 1" (2.5 cm) below the top of the jar by means of the wire wrapped around the neck of the vial and bent to form a hook that fits over the lip of the jar.
- (5) To activate the unit either 6 ml of ethyl acetate or 3 ml of methyl butyrate are placed in the vial, and approximately 100 ml of tap water added to the jar. Either of these chemicals is effective until it evaporates; usually each persists for five days. Both ethyl acetate and sodium butyrate are equally effective but the former is cheaper.