EXOTIC AEDES SURVEILLANCE
OVITRAP SERVICING PROCEDURES

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1.0 INTRODUCTION

Ovitraps are special egg traps used to detect the presence or importation of dengue and yellow fever vector mosquitoes such as *Aedes aegypti* and *Aedes albopictus*. *Aedes aegypti* is absent from all States in Australia except Queensland (Sinclair 1992), while *Ae. albopictus* is not present but poses a real threat (Kay et al. 1990, Whelan 1981, Whelan 1991).

Ovitraps consist of a water filled blackened jar with a porous paddle dipping into the water. These jars are particularly attractive egg laying locations for artificial container breeding mosquitoes such as *Aedes* (Stegomyia) mosquitoes. They are usually situated at favourable sites around vulnerable points of entry such as international airports or seaports. However for States such as the NT, WA and NSW, where importation of *Ae. aegypti* can occur from Queensland, the vulnerable entry points include domestic airports and shipping facilities, tyre businesses, caravan parks, plant nurseries with interstate connections, and interstate transport companies and removalists. Those towns with direct road, sea or air connections to Queensland are vulnerable, with the highest potential occurring at sites closest to Queensland, or with the most traffic.

An ovitrap program can be a relatively cheap method to detect exotic *Aedes* importation. However it is a detection method for an event that may happen with very low frequency. There may be many years of negative results except the recovery of domestic species already present. This can lead to complacency and a reduction in priority or allocation of resources. However it may only need one interception in 10 or 20 years of operation to prevent the establishment of an exotic mosquito species.

An ovitrap program can only be useful if it is a thorough and continuous operation, with weekly or fortnightly servicing, and with people who can competently rear and identify mosquito larvae. There should also be a regular evaluation of the procedures and the results of the program. If ovitraps are operated effectively and efficiently, they can be early warning indicators for timely eradication procedures. Timely eradication can ensure the various States remain free of exotic species of *Aedes* mosquitoes and certain mosquito borne diseases.

2.0 EQUIPMENT REQUIRED

The equipment required includes:

**Field equipment**

- 1 litre black painted glass jars or clear glass jars inside black plastic casing containers (ovitrap jars)
- 3 sets of numbered 'masonite' paddles - approx 16 cm long and 3 cm wide, with stainless steel wire hooks attached to one end
- A bottle of dechlorinated water (tap water left to stand in containers). These are filled after each use and set aside for the following week.
- Results sheet or record book
- Observation form (to record any disturbance to ovitrap)
- Maps with exact locations of ovitraps
- Jar carry basket

Laboratory equipment
- Rearing trays (12 x 17 x 4 cm)
- Ground fish food.
- A supply of dechlorinated tap water
- Labelled larval storage jars
- 70 % alcohol
- Microscope and associated equipment
- Larval identification keys
- Results and summary record sheets

3.0 THE OVITRAP

The ovitrap consists of a 1 litre blackened screw top glass jar (with lid) and a masonite paddle (15 x 2 cm). The paddle is suspended vertically by a stainless steel hook onto the inside of the jar with the rough side facing outwards. Each jar, paddle, screw on lid and black plastic casing for the ovitrap jar is numbered with an identical number and each ovitrap has a different number. Three sets of numbered paddles are used on a rotational basis. One set is in the ovitrap, one set is being hatched and the other set is being sterilised and dried.

4.0 OVITRAP POSITION SELECTION

Ovitrap positions at vulnerable sites should be relatively secluded, shaded, low to the ground, near vegetation and protected from rain and animal disturbance. They should also be adjacent to areas where there is regular human activity. Female Aedes (Stegomyia) mosquitoes are generally attracted to humans for blood meals and require a nearby egg laying site after feeding. Ovitraps can be placed between two bricks or stones, or behind or under a suitable object such as a wash trough if they are prone to disturbance. If animals drink from the ovitraps, a wide mesh screen can be placed over the ovitrap, but the mesh should be no smaller than 2 cm to prevent hindrance to mosquito entry. The ovitrap should not be placed in a position with spider webs or inside very thick vegetation.

5.0 PREPARATION OF OVITRAPS FOR PLACEMENT

Ovitraps are prepared in the laboratory or special preparation room.

1. Fill the clean ovitrap jars with dechlorinated water to depth of 10 cm (approx. 650 ml).
2. Place the numbered clean masonite paddle into the corresponding ovitrap jar and screw on the lid.
3. Put the ovitrap jar into corresponding black plastic casing.
4. Load the ovitraps into the carry baskets ready for transportation to the sites.

6.0 OVITRAP PLACEMENT

The new ovitraps are transported to the selected ovitrap positions.

1. New ovitraps are positioned at the same time as exposed ovitraps are collected.
2. At the trap site unscrew the lid and hook the masonite paddle over the lip of ovitrap jar.
3. Ensure that the ovitrap jar, masonite paddle and black plastic casing have corresponding site numbers.
4. Ensure that the water in the jar is at the correct level and top up if required.
5. Place the ovitrap in the designated position.

7.0 OVITRAP COLLECTION

Exposed ovitraps are collected after the new ovitraps have been positioned.

1. Unhook the paddle and lower it into the jar.

2. Screw on the lid, (from the new ovitrap just positioned), to the exposed ovitrap to be collected. Make sure that the lid is screwed on tightly.

3. Recorded any disturbances to the ovitrap on the observation form (eg. invasion of ovitrap by ants or frogs, ovitrap stolen or vandalised, no water left in ovitrap jar, or jar tipped over).

4. Collect the exposed ovitrap and record the presence of larvae or pupae on the observation form.

5. Return collected ovitraps to the laboratory.

6. Any relocation of the trap position should be recorded on the results form and the field map.

The presence of any pupal skins indicates that inspections have been too far apart and the ovitrap servicing period should be shortened. This aspect should be brought to the attention of the supervisor immediately on return to the laboratory.

8.0 OVITRAP PROCESSING

All rearing should be done in a secure mosquito proof room or an insectary. The insectary should have warning signs on the entry and there should be restricted access. No insecticides should be used in the insectary and contact with insecticides should be avoided prior to entering the insectary.

Immediately on returning from the field:

1. Remove the paddle from the ovitrap and lay it out on clean paper towels (rough surface up) to dry for three days, in an ant free environment (use water baths or ant traps). Ensure that the paddles do not touch each other.

2. Tip water from ovitrap jar into a rearing tray (12 x 17 x 4 cm) that is marked with trap site number and the date of collection.

3. Remove any 4th instar larvae and place them into a collection vial that contains 70 % ethanol. Label the vial with the trap site number and the collection date.

4. If any live pupae are found, place them in a small container with water from the collected ovitrap (eg plastic urine sample container). Label them with the trap site number, date of collection and the number of pupae recovered. Place this container in a separate rearing container or cage. Check for adult emergence every day. Maintain the adults alive for approximately 24 hours after emergence, and then kill them by freezing for 10 minutes. Identify the adult mosquitoes and record the identifications on the record form.

5. Reared adults of common domestic species can be discarded. New or exotic species must be pinned and labelled by standard procedures and stored adequately in insect storage boxes or cabinets.

6. Collect any larval or pupal skins in the ovitrap and place in a vial with 70 % ethanol. Label the vial with the trap site number and the collection date. The larval skins can be used for identification.

7. Record on the results sheet if any larvae or pupae were found in the ovitrap jar.
8. Repeat for each ovitrap.

9. After three days the dry paddles are placed in numbered rearing trays and fully immersed in dechlorinated water for 7 days.

10. Glass jars are examined by eye for mosquito eggs, dried for 3 days, flooded to the top and left for 1 week. This will allow hatching of any eggs on the side of the glass jar. If eggs were observed on the glass, sufficient food is placed in the jar when flooded, and observed daily for hatching. If no eggs are observed, a small amount of food is placed in each jar in case there are eggs present.

11. Any larvae hatched in the jars are placed in the corresponding rearing tray (see above). If there is no hatching, the jars are sterilised by boiling water and washed in the dishwasher in preparation for reuse.

9.0 LARVAL REARING

1. Larval rearing should be carried out in an unairconditioned mosquito proof room or insectary. The room will require adequate bench space for larval rearing trays. Each larval rearing tray may contain larvae over a 2 to 3 week period. If larval growth takes longer than 3 weeks, it may be necessary to raise the temperature of the room (oil heaters are useful).

2. Rearing trays are inspected every day after flooding of the paddles. Any dead larvae and 4th instar larvae are removed by pipette and transferred to a labelled vial of 70% ethanol. Cross contamination between trays should be avoided by washing the pipette between each procedure.

3. Hatched larvae are reared in the rearing tray under a hygienic feeding program. The larvae are fed ground fish flakes (EXCELPET - Tropical Fish Food is satisfactory). The ground fish flakes are soaked in water for one hour and the suspension added with a pipette below the water surface to reduce bacterial problems on the water surface.

4. Food should be added at Day 1 to aid hatching and ensure food availability for newly hatched larvae. Additional food should be added as required during larval growth. Care must also be taken not to add too much food or to put fingers in the water as this can promote bacterial growth (evident by the presence of a white scum on the surface of the water). Any accumulation of old food should be removed with a pipette, avoiding cross contamination between rearing trays.

5. If any larvae appear different from *Ae. notoscriptus* or other common domestic species, a sample should be identified immediately.

6. The larvae in the trays are collected when they die or when they reach 4th instar stage. The larvae are identified with the aid of a microscope and larval identification keys, and the number of larvae of each species are counted.

7. Common domestic larvae can be discarded after identification. Exotic species should be stored in 70% alcohol in labelled vials and placed in a larval collection or reference facility. The identification of exotic larvae should be confirmed by a specialist taxonomist.

10.0 CLEANING OF OVITRAPS

1. After one week, paddles are removed from rearing trays, scrubbed clean to remove any old hatched eggs and scum, sterilised in boiling water and dried. When sterilising the paddles make sure all surfaces of the paddle are exposed to the boiling water.

2. Once all the larvae have been reared to 4th instar stage, or at the end of the 2 or 3 week rearing period, rearing trays are sterilised by boiling water and washed in a dishwasher.
11.0 RECORDING AND REPORTING OF RESULTS

1. Results of the identifications and numbers recovered from each ovitrap should be recorded on the results form at the end of each rearing period, and entered into a database or another recording method.

2. Any possible exotic mosquito species should be brought to the attention of the supervisor immediately.

3. Confirmed identifications of exotic species should be reported to the relevant authorities and they should be advised of the possible need to initiate immediate eradication procedures (see ‘Exotic mosquitoes arriving on overseas vessels’).

4. The yearly results should be tabulated in summary sheets. These should include the number of inspections, the number of times the ovitrap was positive, the number of larvae present, and the identity of all larvae. The results should be evaluated for under performing sites. Under performing sites should be considered for relocation unless they are close to high risk or demonstrated entry points.

12.0 CONCLUDING REMARKS

These procedures have been developed to enable the rapid detection of exotic *Aedes* larvae. They require a high degree of consistency and accuracy to be efficient and are by necessity long term programs requiring adequate allocation of resources. Ovitrap programs should be carried out with a corresponding source reduction program around the vulnerable sites to improve the likelihood of detection of importations. Once an exotic species has been detected and the identification confirmed, there should be a rapid and organised response, which will usually involve eradication procedures. If importations are detected rapidly, eradication measures have a high likelihood of success. An efficient *Aedes* ovitrap program can help to maintain Australia free of additional exotic mosquitoes and certain mosquito borne disease outbreaks.

13.0 REFERENCES


