

ESTABLISHMENT AND MAINTENANCE OF A COLONY OF THE OLD WORLD SCREWORM FLY, *CHRYSOMYA BEZZIANA* AT BALITVET IN BOGOR, WEST JAVA, INDONESIA

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ABSTRAK

SUKARSIH, SUTIJONO PARTOUTOMO, ROBERT TOZER, EDY SATRIA, GENE WIJFFELS, dan GEORGE RIDING. 2000. Pembangunan dan pemeliharaan koloni lalat *the Old World Screwworm Chrysomya bezziana* di Balitvet, Bogor, Jawa Barat, Indonesia. *Jurnal Ilmu Ternak dan Veteriner (Edisi Khusus)* 5 (3): 144-149.

Koloni lalat *the Old World Screwworm Chrysomya bezziana* telah dibangun dan dipelihara di Balai Penelitian Veteriner, Bogor, Jawa Barat, Indonesia, sejak 1994. Kultur lalat yang sesuai dan diet yang dipilih yang mampu mempertahankan daya reproduksi lalat dalam jangka panjang diuraikan dalam tulisan ini. Di samping itu, kelangsungan hidup jangka panjang dari koloni lalat dipertahankan dengan teknik pengkulturan yang efektif dan dapat diandalkan, sedangkan keragaman genetik dan ketahanan hidup koloni lalat dipertahankan melalui perkawinan reguler antara lalat yang dikultur dan lalat liar yang diperoleh dari lapangan. Koloni lalat tersebut telah digunakan sebagai sumber penunjang bahan-bahan dari larva lalat yang dipakai untuk mengisolasi dan mengidentifikasi antigen protektif pada proyek vaksinasi lalat *screwworm* yang dibiayai oleh ACIAR. Larva lalat telah dipakai untuk pengembangan dan pemakaian secara rutin teknik evaluasi efikasi vaksin. Koloni lalat secara terus-menerus tetap berguna baik sebagai sumber pembuatan vaksin lalat *screwworm* maupun penelitian baru yang lain, di antaranya termasuk penelitian baru tentang evaluasi zat pengikat lalat *screwworm*.

Kata kunci: *Chrysomya bezziana*, kultur, koloni, larva, vaksinasi

ABSTRACT

SUKARSIH, SUTIJONO PARTOUTOMO, ROBERT TOZER, EDY SATRIA, GENE WIJFFELS, and GEORGE RIDING. 2000. Establishment and maintenance of a colony of the Old World Screwworm fly *Chrysomya bezziana* at Balitvet in Bogor, West Java, Indonesia. *Jurnal Ilmu Ternak dan Veteriner (Edisi Khusus)* 5 (3): 144-149.

A colony of the Old World Screwworm fly, *Chrysomya bezziana*, has been established and maintained at Balai Penelitian Veteriner, Bogor, West Java, Indonesia since 1994. Culture on a convenient, defined diet able to sustain long term reproducibility of insect performance is described. The long-term viability of the colony has been further assured with an effective and reliable culturing procedure, with the genetic diversity and robustness of the colony being maintained through regular infusions of wild-type field isolates. The colony has been a reliable source of larval material used for the isolation and identification of protective antigens in the ACIAR funded Screwworm Fly Vaccination Project. Larvae have been used in the development and routine application of assays employed to evaluate vaccine efficacy. The colony continues to be a valuable resource for both screwworm fly vaccine and other new research including the evaluation of screwworm fly attractants and lures.

Key words: *Chrysomya bezziana*, culture, colony, larvae, vaccination

INTRODUCTION

The Old World Screwworm fly, *Chrysomya bezziana*, is a cutaneous myiasis-producing parasite infesting a wide variety of wild and domestic animals and man. Its distribution includes tropical and subtropical regions of Africa, Asia and South-east Asia including Indonesia (SIGIT and PARTOUTOMO, 1983; SUKARSIH *et al.*, 1989), Papua New Guinea (NORRIS and MURRAY, 1964; SPRADBERY *et al.*, 1976) and Malaysia (SPRADBERY and VANNIASINGHAM, 1980; PARAMESWARAN *et al.*, 1994). Both the threat to human

and animal health and the economic costs associated with the control of *C. bezziana* are considerable. The need for research into the efficient control of this parasite is well documented (WILLADSEN, 2000).

A number of independent events helped initiate the establishment of a screwworm fly research facility at Balitvet, Bogor, Indonesia. These events included: (a) the closure of the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Division of Entomology, Screwworm Fly Unit in Boroko, Papua New Guinea (SPRADBERY, 1992); (b) the concerns of the Australian Meat and Livestock Research and

Development Corporation (AMLRDC) relating to the ever present screwworm fly threat to the northern Australia livestock industries (a concern later supported by AMLRDC research project No. CS 103 in Bogor) and (c) the initiation of a collaborative ACIAR project between CSIRO Tropical Animal Production, Balai Penelitian Veteriner (Balitvet), Bogor and Institut Teknologi Bandung (ITB). The ACIAR project's main objective was to investigate the feasibility of producing a vaccine to control *C. bezziana*. The project's principal need for a *C. bezziana* colony was to provide fly larvae for the isolation of antigens and for the development of assays to evaluate vaccine efficacy.

In nature, screwworm fly larvae can only survive and mature on fresh, healthy tissue, usually a wound site. The wound becomes larger and deeper through the lacerating action of larval mouth hooks and successive fly strikes (SPRADBERY *et al.*, 1976). To reproduce similar growing conditions in the laboratory was a challenge. This paper describes the successful establishment and continued maintenance of a *C. bezziana* colony in the laboratory.

MATERIALS AND METHODS

Source of larvae for establishment of the colony

The original Balitvet-Bogor screwworm fly colony was first established in June 1994. Fly larvae were sourced from a naturally infested wound on a merino sheep housed at the Balitvet Cimanglid facility, fifteen kilometers from Bogor, West Java. The animal was slaughtered and approximately fifty, second and third instar larvae were extracted from a deep multi-struck wound. The second instar larvae were reared to

pupation in a crude meat medium. The emergent flies became the nucleus breeding population of the Balitvet-Bogor screwworm fly colony.

Screwworm fly larval growth media

The original larvae collected in the field were grown on a crude meat medium (SUKARSIH *et al.*, 1989), Table 1(a). A new improved larval rearing media (LRM), Table 1(b), was introduced into the colony in 1995 and, with only minor modifications, has been used successfully to this day.

Larval rearing procedure

The rearing of screwworm fly larvae was performed in a room dedicated to the purpose with the temperature held at 30-32°C and the relative humidity at 75-80%. The procedure for the rearing of larvae from eggs to pupae is shown schematically in Figure 1 and described as follows:

Day 1. Fly eggs were introduced into a batch of fresh meat-blood mixture (MBM). The MBM was prepared by mixing 250 ml of minced bovine meat (blended to a paste in a household blender) with 30 ml of fresh bovine blood collected in ethylenediamine tetraacetic acid (EDTA) disodium salt. The MBM (50 ml) was placed in the corner of a plastic hatching tray (18.5 cm × 13.5 cm × 4.5 cm deep) and 125 mg of fly eggs (oviposited the previous evening) were introduced to the MBM. Fresh LRM (50 ml) was placed beside the MBM containing the eggs. The tray was covered with gauze, half covered with a damp towel and incubated overnight.

Table 1. Screwworm fly larval rearing media

Larval growth media	Ingredients	Amount	Supplier
(a) Original meat diet	Lean minced beef	54%	Local
	Whole blood	15%	Local
	Water	30.8%	
	Formalin	0.2%	AR grade
(b) Larval rearing media (LRM)	Spray dried bovine blood	60 g	Californian Spray Dry Co. CA.
	Low-fat milk powder	30 g	Local
	Whole egg powder	30 g	Sunny Queen Products, Brisbane, Australia.
	Water Lock [®] superabsorbent polymer gel	12 g	Grain Processing Corp. Muscaline, IA.
	Formalin	1 ml	AR grade
	Water	860 ml	

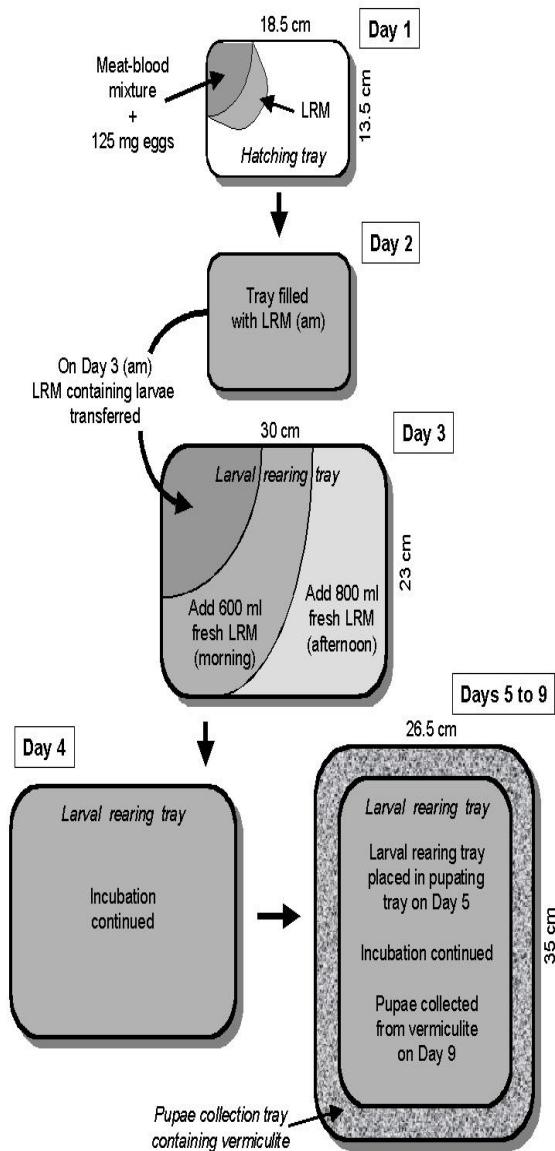


Figure 1. Larval rearing procedure. The flow diagram shows the series of larval rearing events from introduction of eggs on the morning of Day 1 to the collection of pupae on Day 9

Day 2. Freshly hatched larvae migrate from the MBM to the LRM overnight. In the morning the trays were filled completely with LRM and incubation was continued.

Day 3. In the morning, LRM containing the larvae was transferred from the hatching tray to a corner of the larval rearing tray (30 cm × 23 cm × 4.5 cm deep) and 600 ml fresh LRM was again placed beside the infested LRM. Incubation was continued. In the afternoon, a

further 800 ml of fresh LRM was added to the larval rearing tray. By the time filling of the tray was complete, the media was approximately 3.0 cm deep. Following addition of fresh LRM, incubation conditions were continued through to day five.

Day 5. On day five, the larval rearing tray was placed into a pupae collection tray (35 cm × 26.5 cm × 4.5 cm deep) which was half filled with blended vermiculite (particle size < 2 mm dia.). During the next three days larvae crawled out of the LRM and pupated in the vermiculite. Incubation was continued.

Day 9. The vermiculite containing the pupae was passed through a sieve (2 mm). The pupae were collected and stored at room temperature (RT).

Fly emergence and feeding

Days 9-12. On day nine, 25 g of pupae were placed in a small tray inside a fly hatching cage (46 cm × 30 cm × 25 cm) and incubated (RT) in the fly hatching room. The hatching room was fly screened and darkened by placing UV car window film, 40% light transmission, over the windows. By day 12, most of the flies had emerged (91.5% -96.0%).

Days 13-15. Flies were fed on minced bovine meat, offered fresh each day for three days.

Oviposition

After larval evacuation from the LRM on day nine (large tray, Figure 1), the spent media was mixed 1:1 with water, passed through a coarse sieve (1.4 mm) then a fine gauze. The liquor, oviposition medium (OPM), was stored in a plastic bottle (RT) and allowed to mature for 24 - 36 h, then stored at 4°C until required.

Day minus 1. The OPM was equilibrated at RT. New eggng boards were impregnated with OPM overnight at RT.

Day 1. Previously used eggng boards were dipped in OPM, then all boards were allowed to drain and air dry at RT for afternoon use. Oviposition devices (Figure 2) were prepared with the dried eggng boards protruding approximately 1 cm above the OPM soaked sponges. (Note: The volume of LRM used in the oviposition devices was approximately 400 ml. This volume kept the apparatus well wetted throughout the eggng period, preventing the eggs from drying out.) In the afternoon, oviposition devices were placed in the fly cages. Gravid adult female flies are attracted to the OPM odour and oviposit on the boards overnight (Figure 2).

Day 2. In the morning, the freshly laid eggs were harvested by spatula from the eggng device and stored in a humid container, ready for immediate use.

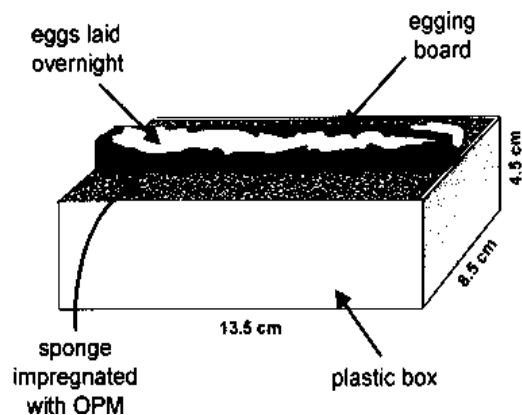


Figure 2. Schematic diagram of an oviposition device used for the collection of *C. bezziana* eggs. Gravid female flies are attracted to the egg board by the OPM odour and deposit their eggs on the egg board overnight

Infusion of “wild” strain flies into the colony

To maintain viability and vigour within the colony, screwworm fly larvae were collected from naturally infested wounds in the field and incorporated into the Balitvet colony at least once per year. The larvae were raised in media as previously described, then allowed to develop for three generations as a wild population. Following this, wild males were then mated with an equal number of colony females. Larval progeny of this mating were reared as previously described. Subsequent generations of this line became the new colony and flies of the old colony were destroyed.

RESULTS AND DISCUSSION

Screwworm fly larval growth media

The original wild-type larvae and the subsequent generations used to establish the Balitvet screwworm fly colony were initially grown on a crude meat medium (SUKARSIH *et al.*, 1989; see Table 1). The larvae did grow in this medium, they reached pupation, and emerged into viable flies. However, there were many inherent problems with the use of this media. Details of the major problems follow. Firstly, larvae buried themselves in the crudely minced meat tissue present in the media and were difficult to locate and recover. Larvae were often transferred to fresh media one by one. This process was time consuming and made it difficult to expand the size of the colony sufficiently to meet demands for larger quantities of material.

Secondly, the crude media was easily contaminated with bacteria and putrefied rapidly, despite the addition of formalin to 0.2%. The medium thus needed to be changed twice a day. Thirdly, the supply and quality of minced beef was variable. The cost of beef had the potential to fluctuate and at times was prohibitively expensive compared with dried blood, as was used in the subsequent LRM diet (Table 1). Finally and most importantly, pupal weights tended to be low (27.9 ± 1.8 mg, Table 2), resulting in adult flies with poor fertility and limiting the long-term viability of the colony.

A new improved larval rearing media, Table 1, was introduced in September 1995, using a formulation similar to that developed by TAYLOR and MANGAN (1987) and TAYLOR *et al.* (1991), for the rearing of New World Screwworm fly, *Cochliomyia hominivorax* larvae. A similar medium was used successfully with *C. bezziana* at the Screwworm Fly Unit in Boroko, in Papua New Guinea (TOZER, unpublished). The new media improved culturing conditions considerably, not only for the growth of larvae but also for the convenience and comfort (less bad odours) of the staff working in the colony rearing rooms. Pupal weights, depending on the source of dried blood in the media, increased by up to 42%. Three different sources of spray dried bovine blood were trialed. Between September 1995 and July 1996, two batches of dried bovine blood were sourced from Brisbane, Australia. One batch proved to be better than the other in terms of pupal weight, (Brisbane batch 1, 33.0 ± 1.9 mg and Brisbane batch 2, 25.8 ± 2.4 mg, Table 2). Californian spray dried bovine blood (Californian Spray Dry Co., CA.) was introduced in July 1996 and has been used to date. Pupal weights were consistently high, and have remained comparable to 1996 data, 39.7 ± 2.9 mg, (Table 2) to this day.

Colony maintenance

The Balitvet *C. bezziana* colony is maintained essentially as a closed culture depending on continuous generation cycles. Larvae are routinely grown to pupation as described in the Material and Methods. From 125 mg eggs in each hatching tray batch, approximately 1300 larvae reach pupation.

From each 25 g of pupae placed in individual hatching cages, approximately 600-650 flies emerged. Fly emergence efficiency from batches assessed over a period of time was between 91.5 and 96.0%. Once the flies have been fed on a meat diet for several days and mating has occurred, gravid female flies are ready for oviposition. Egg laying occurs overnight on a specially prepared oviposition device (Figure 2), placed in the cage for the purpose. Fly eggs are used for the supply of larvae for vaccine experiments as well as the production of the colony's next generation.

Table 2. Effect of diet on pupal weights

Larval rearing diet	Total number of pupae (rearing tray batches)	Mean weight of pupae (mg) ($\bar{x} \pm SD$)
Original meat diet	2,443 (4) ^a	27.9 \pm 1.8
Larval rearing media (LRM)		
(a) Dried blood – Brisbane #1	15,123 (10)	33.0 \pm 1.9
(b) Dried blood – Brisbane #2	15,205 (25)	25.8 \pm 2.4
(c) Dried blood - Californian	15,568 (17)	39.7 \pm 2.9

^a The weighing of pupae was not a routine activity. The weight of pupae from larvae grown on the original meat diet was only sampled just prior to the introduction of the new LRM, hence the low number of trays measured

Supply of experimental material

The Balitvet Screwworm fly facility has proven to be a reliable source of larvae for experimental purposes. In the early stages of the Screwworm Fly Vaccine Project there was a requirement for both 1st and 3rd instar larvae for vaccination trials. By scaling up the larval rearing procedure, these production targets were readily achieved. At the same time, assays were being developed for use in the assessment of vaccination efficacy. Freshly hatched first instar larvae were and continue to be supplied for both *in vitro* and *in vivo* assay systems (PARTOUTOMO *et al.*, 1998; SUKARSIH *et al.*, 2000). Screwworm fly peritrophic membrane (PM) has become an important source of potential antigens for vaccination trials. *C. bezziana* larvae supplied from the colony were used in the production of larval PM under sterile culture conditions (RIDING *et al.*, 2000).

CONCLUSION

The large scale and infrastructure-intensive nature of the facilities used to rear Old World and New World Screwworm flies can create the impression that culture may be a major undertaking requiring highly specialized facilities. This paper demonstrates that this is not so, and that an inexpensive facility is able to maintain a colony of *C. bezziana* indefinitely with production of insects sufficient for a full research programme. With the incorporation and continued use of an improved larval growth medium in the larval growing cycle, the periodic infusion of wild type flies and the expertise gained by facility staff, the fertility and long-term viability of the Balitvet Screwworm colony is assured. Continued financial support for the operation of this valuable resource is essential and should be considered a priority for both the Indonesian government and international animal research agencies alike.

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