Evaluation for the larval and pupal growth conditions for *Chrysomya bezziana* under clinical microbiology laboratory setting in Hong Kong

River C.W. Wong, D.C. Lung, K.M. Chan and T.L. Que

Department of Clinical Pathology, Tuen Mun Hospital, Hospital Authority, Hong Kong Special Administrative Region, China.

Address for correspondence:

River C.W. Wong , Department of Clinical Pathology, Tuen Mun Hospital, Tsing Chung Koon Road, Tuen Mun, Hong Kong SAR, China. E-mail: wcw372@ha.org.hk

Abstract

The Old World screwworm fly (OWS), *Chrysomya bezziana* (Diptera: Calliphoridae) is an obligatory parasite that serves as the major causative agent for traumatic human myiasis in the tropics and sub-tropics of the Old World. This study was conducted to evaluate the larval and pupal growth conditions for OWS from Hong Kong under a typical clinical microbiology laboratory setting without application of extra resources. Thirty-two live larvae were collected from a single patient who suffered from OWS myiasis. The larvae and subsequent pupae were allowed to develop under controlled laboratory conditions at temperatures of 25^0C, 30^0C & 37^0C with a food supply of horse blood agar block or cooked meat medium. This study evaluated for the first time growth conditions for larvae and pupae of a Hong Kong strain of OWS and demonstrated that it is possible to rear larvae through to adult flies by using simple equipments available in a microbiology laboratory. It is possibly best to use 30^0C because least mortality rate for larvae was found at that temperature (10% over all foods, 1 out of 10 larvae) compared to 25^0C (20% over all foods, 2 out of 10 larvae) and 37^0C (42% over all foods, 5 out of 12 larvae) and both horse blood agar block and cooked meat medium could be applied for the feeding of larvae. Pupae were developed into adulthood earlier at 30^0C than 25^0C and the average time duration for pupae completed development was 6.6 days at 30^0C compared to 9.4 days at 25^0C. The findings provide useful information for the rearing of OWS removed from wounds, from larvae to adulthood under laboratory conditions. They facilitate the study of OWS biology and enhance the ability to accurately identify OWS as a causative agent of human myiasis, because identification can be based on larvae as well as adults.

Key words: Old World screwworm, *Chrysomya bezziana*, larvae, pupae, human myiasis, temperature, Hong Kong

Introduction

The Old World screwworm (OWS), *Chrysomya bezziana* (Diptera: Calliphoridae) is one of the most important causative agents for human myiasis in the tropics and sub-tropics of the Old World. It is an obligatory parasite and develops on live hosts. Ng et al, from our department reported the first human infestation due to *Chrysomya bezziana* in Hong Kong, a case of oral myiasis.[1] According to the record of human myiasis cases from the Centre for Health Protection, nine cases were reported from Hong Kong in 2003, eight cases in 2004, six cases in 2005 and fourteen cases in 2006.[2]

The life cycle of OWS starts when adult females lay their eggs at the dry edges of a patient's wounds or in body orifices such as the ear, nose and oral cavity.[3] First instar larvae hatch after 18 - 24 hours and develop into second instar after 12 - 18 hours then further moult into third instar about 30 hours later. Larvae infest the wound for 3 - 4 days and then drop to the ground and pupate. Adult flies will emerge from the pupa after 7 days.[4]

Clinical diagnosis of human myiasis is dependent on the discovery of dipterous larvae (maggots) from the patient's wound and laboratory diagnosis is based on identification of the larvae collected. It is helpful to allow some larvae to feed and develop into third instars in the laboratory, or to pupate and emerge as adults, in order to ensure development of mature identification features, that can help in the accurate identification of different species that cause human myiasis. However, it is usually considered difficult to rear obligatory parasites such as the larvae of OWS to maturity. Therefore, this study aimed to determine the most simple and appropriate growth conditions for larval and pupal development of OWS under the conditions of an existing clinical microbiology laboratory in an acute hospital of Hong Kong, without application of extra resources, by examining the effect of temperature (25^{0}C, 30^{0}C & 37^{0}C) and 2 types of food supply (horse blood agar & cooked meat medium).

Materials and methods

Source of larvae

Thirty-two live larvae were collected from a foot wound of a single patient who suffered from myiasis and was admitted to our hospital. Larvae were identified as OWS according to published identification keys.[4]

Maintenance of living larvae

Horse Blood Agar (BA)

Columbia agar (Oxoid Ltd., UK) supplemented with 5% horse blood, which was originally designed for bacteriological culture, was prepared for feeding to larvae. Live larvae were placed into a transparent plastic sputum bottle with a perforated lid in order to allow gaseous exchange. Five to six blocks of blood agar (1 cm x 1 cm) were put inside the bottle and incubated at one of three temperatures (25^{0}C, 30^{0}C or 37^{0}C) from Day 1 to 4.

Robertson Cooked Meat Medium (RCM)

For each larval container, a total of 1.5 gram of cooked meat medium (Oxoid Ltd., UK) was first softened by thioglycollate fluid and autoclaved at 121^{0}C for 15 minutes and then cooled to room temperature. Live larvae were incubated with dried RCM as above.

Completion of life cycle

In the first stage of the experiment (Day 1 to 4), the live larvae were feed on either BA or RCM inside a plastic bottle and larvae were being cleaned by immersed into

70% ethanol for 1 minute on each day. Food supplies were removed and replenished on each day. At the end of Day 4, all the larvae from different temperatures were transferred into two-sealed individual transparent plastic boxes (15 cm x 11 cm x 5.5 cm) with a plain petri dish of mixed food supply (BA & RCM) provided. In this second stage of experiment, several pieces of leaf were placed around the petri dish. These plastic boxes were incubated at 25^0C or 30^0C respectively. From Day 5 to 14, the boxes were examined for pupal formation on each day. Pupae were transferred individually to new plastic sputum bottles sealed by gauze and incubated at the same temperature and examined for adult emergence daily.

Results & Discussion

In the first stage of the experiment, 30^0C has the least mortality rate for larvae (10% over all foods, 1 out of 10 larvae) compared to 25^0C (20% over all foods, 2 out of 10 larvae) and 37^0C (42% over all foods, 5 out of 12 larvae) and both horse blood agar block and cooked meat medium could be applied for the feeding of larvae. In the second stage of the experiment, pupae were developed into adulthood earlier at 30^0C than 25^0C as the average time duration for pupae completed development was 6.6 days at 30^0C compared to 9.4 days at 25^0C. Leaves were supplied as a natural site for pupation. Sawdust is commonly used as a pupation medium in insect cultures, but for irregular rearing, leaves were found to be much easier to collect and inexpensive. OWS is widely distributed around the tropical and sub-tropical region of the Old World.[3,5] Hong Kong has sub-tropical climates and the average temperature during summer is around 30^0C. To conclude, among the temperatures examined in this study, 30^0C was found to be most appropriate for larval and pupal development on the two medium tested.

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Table 1. Comparison between different growth conditions for the development of *Chrysomya bezziana* larvae and pupae under laboratory conditions

	Day 1	Day 2	Day 3	Day 4		Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Blood agar					Blood agar & Cooked meat										
25^0C	L x 5 [a]	L x 5	L x 3(2) [b]	L x 1	25^0C	L x 8	L x 2(1)	P x 12	P x 12	P x 12	P x 12	A x 2 [d]	A x 2	A x 2	A x 10
				P x 2 [c]		P x 5	P x 10					P x 10	P x 10	P x 10	P x 2
30^0C	L x 5	L x 5	L x 4(1)	L x 4	30^0C	L x 6	P x 11	P x 11	P x 11	P x 11	A x 4	A x 9	A x 9	A x 9	A x 9
						P x 5					P x 7	P x 2	P x 2	P x 2	P x 2
37^0C	L x 7	L x 7	L x 7	L x 7											
Cooked meat															
25^0C	L x 5	L x 5	L x 5	L x 5											
30^0C	L x 5	L x 5	L x 5	L x 5											
37^0C	L x 5	L x 5	L x 0(5)												

Remark: [a] L means larvae x no. of live larvae. [b] (no. of larvae died). [c] P means pupae x no. of pupae. [d] A means adult flies x no. of adult flies.
1st stage of experiment (Day 1 to 4) for development of larvae (25^0C, 30^0C & 37^0C).
2nd stage of experiment (Day 5 to 14) for development of pupae into adult flies (25^0C & 30^0C).