Tropical Biomedicine 22(2): 185–189 (2005)

Sterilisation of *Lucilia cuprina* Wiedemann maggots used in therapy of intractable wounds

Mohd Masri, S., Nazni, W.A., Lee, H.L., T. Rogayah, T.A.R. and Subramaniam, S.
Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia.

Abstract. Three new techniques of sterilising maggots of *Lucilia cuprina* for the purpose of debriding intractable wounds were studied. These techniques were utilisation of ultra-violet C (UVC) and maggot sterilisation with disinfectants. The status of sterility was checked on nutrient agar and blood agar and confirmed with staining. The indicators for the effectiveness of the methods were sterility and survival rate of the eggs or larvae. Egg sterilisation with UVC had the lowest hatching rate (16±0.00%) while egg sterilisation with disinfectants showed high hatching rate (36.67±4.41%) but low maggot survival rate (31.67±1.67%). Sterilisation of the maggots was the most suitable, since the survival rate was the highest (88.67±0.88%). Complete sterility was achieved in all cases, except that *Proteus mirabilis* was consistently found. However, the presence of this microorganism was considered beneficial.

INTRODUCTON

Maggot debridement therapy has been long used as a traditional way of cleansing and healing gangrenous wounds and it is said that the Mayan Indians wrapped wounds with a dressing made of sun-exposed beef blood that would pulsate, apparently with maggots, a few days after it was applied (Weil *et al.*, 1933). Napoleon's Surgeon-in-Chief (Baron Dominic Larrey) reported that maggots developing in infected wounds prevented further infection and accelerated wound healing (Goldstein, 1931). The first scientific studies of medicinal maggots uses were conducted by Baer (1872-1931), a clinical professor of orthopaedic surgery at the John Hopkins School of Medicine in Maryland and served in World War I (Thomas *et al.*, 1996). Following the wartime experiences, Baer treated four children with intractable bone infections (osteomyelitis) at the Children's Hospital in Baltimore in 1928 and was very successful as the wounds healed in six weeks (Baer, 1931). Baer began to use the technique more widely, but unfortunately several of his patients developed tetanus and he concluded that it would be necessary to use sterile maggots for future work.

The outer surfaces of fly eggs are usually very heavily contaminated with bacteria and need to be removed or killed if the emerging maggots are to remain sterile (Fine & Alexander, 1934). A technique was developed by Baer with the use of a solution containing mercuric chloride (1:1000), 25% alcohol and 0.5% hydrochloric acid (Baer, 1931). Simmons (1935) reported satisfactory sterilisation using 5% formalin, 1% sodium hydroxide but still did not kill all spore-forming bacteria such as *Clostridium perfringens* or *Clostridium tetanii*. Sterilising hatched larvae was found to be virtually impossible even though some success were reported by Livingston (1932) and Weil *et al.* (1933). The objectives of this study were to develop a new maggot sterilisation technique and to identify microbial contaminants from maggots.

MATERIALS AND METHODS

Fly Colony

The colony of *Lucilia cuprina* used in this study was reared and maintained in the Insectarium of Institute for Medical Research, Kuala Lumpur. Oviposition of the fly was allowed by placing a piece of liver (about 20g) in the cage and left for 7 hours. The complete cycle of *L. cuprina* takes about 3 weeks. The colonies were maintained at 27–29°C, R.H= 70%.

Method of Sterilisation

Three types of sterilisation were used in this study viz., ultra-violet C (UVC), 70 % alcohol and chlorhexidine sterilization.

Eggs sterilisation by UV C

One gram of the eggs was collected, soaked and separated by chlorhexidine and exposed to UVC (wave length 190nm, intensity =180µw-m/cm^2) for every 1 minute intervals, up to 10 minutes. Mortality was also recorded after 10 minutes, while contamination was checked after 24 hours.

Eggs sterilisation by Disinfectants

A total of 1g eggs was collected from the exposed liver for sterilisation. The eggs were soaked in chlorhexidine for 8 minutes followed by 70% alcohol and then rinsed in sterile saline. The eggs were transferred to a medium containing a mixture (1:0.5 w/v) of nutrient agar (Difco) and liver powder. Maggots that appeared after 48 hours were collected and sterilized again with 70% alcohol and stored in a bottle at 10°C. Mortality was also recorded after 10 minutes while contamination was checked after 24 hours in nutrient agar and blood agar.

Maggot sterilisation by disinfectants

Maggots were collected from the cow liver that was previously incubated for 48–60 hours. The late L1 or early L2 maggots were starved for 12 hours and then soaked with chlorhexidine followed by 70% alcohol. Distilled water was used to flush out remaining alcohol, followed by sterile saline. Maggots were introduced into a sterile glass bottle and kept at 10°C. Mortality was recorded after 10 minutes while contamination was checked after 24 hours in nutrient agar and blood agar.

RESULTS AND DISCUSSION

The sterilisation of eggs or larvae to be used in debridement therapy is of paramount importance as they will be applied onto a patient for therapeutic purposes. Thus preventing contaminants such as tetanus reported by Baer (1931) is a priority.

From the results obtained in this study, UVC sterilisation of eggs was not effective even up to 6 minutes of exposure, based on the 24 hours observation time (Table 1). Although longer exposure period of more than 6 minutes allowed complete sterility even after 72h incubation, the hatching rate was practically nil. The surface appearance of most UVC-exposed eggs were blackish and dessicated. The hatching percentage using UVC was very poor. This was due to the effect of UVC on the eggs destroying the embryo. Non-ionising light such as UVC is known to be damaging to insect eggs (Raimon, 1972). In comparison, Beard (1970) reported that house fly eggs are refractory to UV irradiation prior to blastoderm formation and after certain time, eggs showed uniform sensitivity with time. Though sterilisation by UVC showed that bacterial contamination was inhibited after 6 minutes, this technique was not useful practically.

On the other hands, disinfectant-sterilized eggs showed sterility of up to 48 hours while sterilisation of the maggots was effective for up to 24 hours (Table 2). With regards to the percentage of maggot survival after the disinfectant sterilisation (Figure 1), the maggot sterilisation showed higher percentage than the egg sterilisation with 88.67±0.88% larvae survived from a total of 100 larvae sterilized and 19.33±0.67% survived up to seven days whereas only 36.67±4.41 eggs

Table 1. Sterilisation of *Lucilia cuprina* eggs by ultra-violet C

Exposure Time (min)	Post-exposure Time (hours)	Hatching rate (%)	Bacterial Contamination*
1	24	10.00	+
	48	10.00	+
	72	10.00	+
2	24	2.00	+
	48	2.00	+
	72	2.00	+
3	24	0.00	+
	48	0.00	+
	72	0.00	+
4	24	0.00	+
	48	0.00	+
	72	0.00	+
5	24	0.00	+
	48	0.00	+
	72	0.00	+
6	24	2.00	–
	48	2.00	+
	72	2.00	+
7	24	0.00	–
	48	0.00	–
	72	0.00	–
8	24	2.00	–
	48	2.00	–
	72	2.00	–
9	24	0.00	–
	48	0.00	–
	72	0.00	–
10	24	0.00	–
	48	0.00	–
	72	0.00	

* + = with contamination
– = without contamination

Time (min) exposure	Time (hour) post-exposure	Hatching (%) rate	Bacterial contamination*
10	24	14.67±0.88	–
	48	15.33±0.33	–
	72	16.00±0.00	–

hatched from a total of 100 eggs sterilized. From this total, 31.67±1.67%survived but only 4.67 ± 0.33% of the larvae survived up to the fourth day. This indicated that maggot sterilisation provided better chance of maggot survival after the sterilisation procedures. Maggot sterilisation was more viable because maggots are apparently hardier than eggs. The percentage of live and sterile larvae decreased over time since the maggots were not provided with food.

The maggot are usually kept at 10°C so that they become dormant to ensure that they can be stored for longer period. Sterile larvae from eggs sterilisation usually last for 4 days while maggot sterilisation can be stored up to 7 days. Unfortunately, even though sterility was achieved over 24–48 hours, bacterial contaminant was still seen. The microbial contaminant was identified as *Proteus mirabilis*. Greenberg *et al.* (1970) demonstrated that microorganisms ingested by the blowfly *Calliphora vicina* are rapidly eliminated if *P. mirabilis* is also present in the ingested material. Several pathogenic microorganisms have been killed by brief exposure to 15 days old *P. mirabilis* culture broth (Erdmann & Khalil, 1986). Greenberg (1968) referred to the active anti-bacteria constituents as "mirabilicide." It was also reported that the symbiotic relationship between *P. mirabilis* and the screwworm could

Table 2. Sterilisation of eggs and maggots by disinfectants

Observation time (hours)	Egg sterilization	Maggot sterilisation
24	–	–
48	+	–
72	+	–

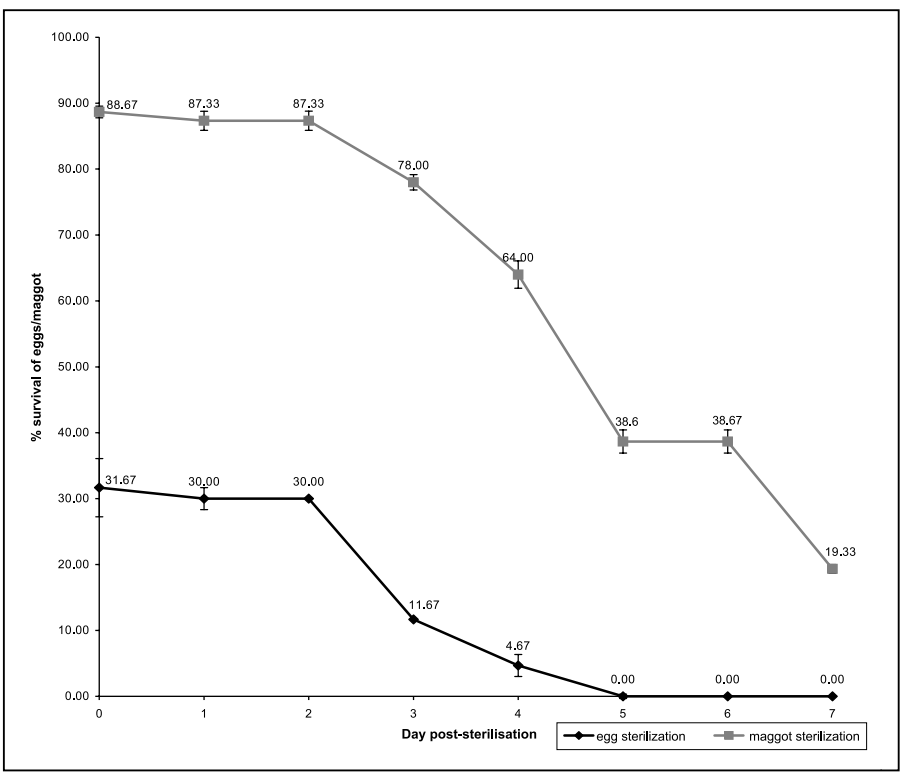

Figure 1. Percentage of maggot survival after sterilisation by disinfectants.

protect the larvae from other harmful bacteria by bactericidal agents secreted by *Proteus mirabilis* (Erdmann & Khalil, 1986). Hence the continued presence of *P.mirabilis* should be considered beneficial.

Acknowledgements. The authors wish to thank the Director, Institute for Medical Research, Kuala Lumpur for permission to publish and staff of Medical Entomology Unit, Institute for Medical Research, Kuala Lumpur for their help.

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