

# Embryonic development of human lice: rearing conditions and susceptibility to spinosad

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*The embryonic development of human lice was evaluated according to the changes in the morphology of the embryo observed through the transparent chorion. Based on ocular and appendage development, three stages of embryogenesis were established: early, medium, and late. Influence of temperature and relative humidity (RH) on the laboratory rearing of *Pediculus humanus capitis* eggs was assessed. The optimal ranges for temperature and RH were 27-31°C and 45-75%. The susceptibility of human louse eggs to insecticide spinosad (a macrocyclic lactone) was assessed by immersion method. The results showed similar susceptibility to spinosad in early, medium, and late stages of head lice eggs. In addition, this study showed similar susceptibility of head and body lice eggs to spinosad, an insecticide that has not been used as pediculicide in Argentina (lethal concentration 50: 0.01%).*

Key words: human lice - embryogenesis - spinosad

Several insecticides have been used against *Pediculus humanus capitis* (Phthiraptera: Pediculidae), a health cosmopolitan pest. Although quite an extensive bibliography about general biology of the human lice eggs is available (Buxton 1946, Kim & Ludwig 1978, Berman & Firstenberg 1979, Hinton 1981, Hatsushika et al. 1983, Ibarra 1993, Kettle 1995, Burkhart et al. 1999 a, b, c), few works about toxicological studies on *Pediculus humanus capitis* and *P. humanus humanus* eggs were reported (Burgess 1999).

The embryonic development is a period involving continuous biochemical, genetic, physiological, and morphological events related to cellular differentiation, growth, and morphogenesis processes (Gilbert 1997). Since these changes are extremely important, the toxicokinetic and toxicodynamic processes will significantly modify during embryogenesis. Toxicological variations during embryonic development have been reported in several species (Tahmisian 1943, Smith & Wagenknecht 1959, Smith & Salkeld 1965, Smallman & Mansingh 1969, Picollo de Villar et al. 1980).

For laboratory bioassay, the age of developing embryo, the optimal rearing conditions, and the methodology for evaluating ovicidal activity, should be standardized. Therefore, the purpose of this work was the optimization of laboratory rearing conditions of louse eggs, and the identification of external characteristics for different stages of development, in order to perform bioassays with insecticides. As an example, human louse eggs were exposed to spinosad by a new immersion method.

## MATERIALS AND METHODS

**Eggs** - Head louse eggs were collected from infested children at the elementary school Guardia de Honor (GH-HL) in Buenos Aires city, where high levels of permethrin resistance were previously reported (Picollo et al. 1998, Vassena et al. 2003).

The head louse eggs were collected from children aged 6-12 years, using a fine-toothed anti-lice comb (Nopucid, Laboratorio ELEA, Buenos Aires, Argentina), according to a protocol approved by ad-hoc committee of the Research Centre of Pests and Insecticides. After collection, the eggs were sent to the laboratory, selected according to the stage of development, and immediately used in the bioassays. For the embryological development studies, freshly laid head louse eggs were obtained from adults previously fed on the arm of a volunteer.

The body louse eggs were obtained from the susceptible colony (S-BL) reared in our laboratory at 28 ± 1°C and 50-60% relative humidity (RH) (Buxton 1946). Freshly laid eggs (< 24 h) were selected and kept at the same conditions until they were used in the test.

**Chemicals** - Technical grade spinosad was from Dow Agro Sciences LLC, Indianapolis, US. Acetone (analytical grade, Merck, Buenos Aires, Argentina), potassium carbonate (K<sub>2</sub>CO<sub>3</sub>) (99%, Sigma, Saint Louis, US), calcium chloride (CaCl<sub>2</sub>) (99.1%, Baker, Phillipsburg, US), lithium chloride (LiCl) (99%, Aldrich, Milwaukee, US), sodium chloride (NaCl) (pure, Parafarm, Buenos Aires, Argentina), silica gel (chrom) (Aldrich) were used.

**Embryological development** - Freshly laid body and head louse eggs were collected after egg laying (< 24 h), were incubated at 28 ± 1°C and 50-60% RH (body lice) and 28 ± 1°C and 75% RH (head lice), and observed daily through a stereoscope microscope Olympus SZ40 until hatching (12 days). The morphological variations of the embryos observed through the transparent chorion were recorded, and the appearance of developing eggs was registered by a digital camera Sony DKA-5000 (3CCD).

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The morphological features were used to determine the developmental stage and selected external markers were used to differentiate between early, medium or late eggs.

**Egg incubation** - The field collected head louse eggs were incubated at different conditions of temperature and humidity in an Ambi-Hi-Low Lab Line environmental chamber (Iowa, US). To control RH, the eggs were kept in closed plastic containers where saturated aqueous solution of different salts or granules of anhydrous silica gel were added. The humidity of the atmosphere in the containers was completely controlled by the level of evaporation of water from the salt solutions or removal of water from the atmosphere by the silica gel. The average of daily RH values measured in the experimental containers, is shown in Table I.

TABLE I

Relative humidity obtained with saturated aqueous solution of different salts

Salt	% RH <sup>a</sup> ± SE	% RH <sup>a</sup> ± SE
	27°C	18°C
H <sub>2</sub> O	99	99
NaCl	76.8 ± 0.3	81.8 ± 0.3
K <sub>2</sub> CO <sub>3</sub>	45.4 ± 0.2	48.8 ± 0.2
CaCl <sub>2</sub>	25.9 ± 0.7	-
LiCl	19.8 ± 0.6	29 ± 1.2
Silica gel <sup>b</sup>	17 ± 0	-

*a*: obtained in closed plastic containers with saturated aqueous solution of different salts; *b*: obtained in closed plastic containers with granules of anhydrous silica gel.

Early, medium or late eggs were incubated at two temperatures (18 and 27 ± 1°C), each one evaluated with increasing percentages of humidity (Table I). Similarly, early, medium or late eggs were kept at 76-82% RH (closed containers with saturated aqueous solutions of NaCl) and increasing temperatures (18, 23, 27, and 31 ± 1°C). The number of emerged nymphs was counted, and those with incomplete emergence were considered as dead. Three replicates of at least three groups of ten eggs were exposed to each one for each humidity/temperature condition; the average percentage of eclosion was calculated and the fiducial limits at the temperature and humidity were estimated.

**Bioassay** - Tests to determine spinosad susceptibility in head and body louse eggs, were done by immersion method. Groups of ten eggs selected according to external markers were fixed to a microscope slide by a double-face adhesive tape. The slides containing eggs were immersed for 10 min in 10 ml of different concentrations of spinosad in pure acetone. Six doses ranging from 0.001 to 0.1% w/v (g/100 ml) were used, and each dose was replicated at least three times. Control eggs were immersed for 10 min in pure acetone. After the exposure, the microscope slides with the eggs were dried on filter paper. Then, head louse eggs were incubated at the optimal conditions assessed in this work for laboratory rearing, and body

louse eggs were kept as previously reported by Buxton (1946). Mortality data of treated eggs were recorded 5 days after the eclosion of controls. The criterium for embryo mortality was eggs with closed operculum or eggs with opened operculum and the insect inside.

**Statistical analysis** - All data were corrected for mortality in the controls (Abbott 1925). Control mortality was always lower than 10%. Three replicates for each concentration were used to obtain a dose-mortality line by probit analysis (Lichfield & Wilcoxon 1949). Lethal concentration 50% (LC<sub>50</sub>) values were expressed as percentage of insecticide weight/volume. Lethal concentration ratio (LCR) and 95% confidence limits (LC) were calculated as described by Robertson and Preisler (1992).

## RESULTS

**Embryological development** - Changes in the appearance of developing embryos of *P. humanus capitatus* and *P. humanus humanus* were assessed at different stages of egg development. The major changes were the visual appearance and darkening of the ocular spots and the visual appearance of the appendages. Pigmented eyes appeared as red spots and turned to black during embryogenesis. The appendages were completely visible at the end of embryonic development.

Based on the colour of the ocular spots and the appearance of the embryo appendages, the developing eggs of head and body lice were divided into three stages: early, medium, and late (Fig. 1). Early eggs were characterized by the absence of external markers, medium eggs showed reddish eyes and appendage outlines, and late eggs showed black eyes and clearly visible appendages (Fig. 1).

**Laboratory egg incubation** - The influence of temperature and humidity on the head louse embryo development was stated for early, medium, and late eggs. No eclosion was observed in any egg incubated at 18°C, so the effect of humidity at this temperature on development stage could not be assessed. Clearly, the continuous exposure of eggs to low temperature (18°C) induced 100% embryo death.

The average percentage of eclosion of early, medium, and late eggs incubated at 27°C and different RH, are shown in Fig. 2. For early embryos, the eclosion increased as humidity increased from 17 to 77%, and abruptly decreased in eggs incubated at 99% RH (Fig. 2). For medium and late eggs, high percentage of hatched eggs was observed at 20, 26, 45, and 77% RH. Emergence was 80, 72, 87, and 86% for medium embryos, and 95, 86, 100, and 100% for late embryos. Low eclosion was observed at 17 and 99% RH in both stages of development (Fig. 2).

The average percentage of eclosion of early, medium, and late eggs incubated at 76-82% RH and different temperatures are shown in Fig. 3. For all, the hatching increased as the temperature increased, showing the highest values from 27 to 31°C. Thus, the optimal laboratory conditions for rearing head louse eggs were 27-3°C and 45-75% RH. According to these ranges, the temperature and humidity finally chosen for the ovicidal test (28°C, 75% RH), was based on practical criteria.



Fig. 1: external appearance of human louse eggs in three different development stages. I: *Pediculus humanus capitis*; II: *P. humanus humanus*. a: early development, b: medium development, c: late development. Ocular spots and appendages are visible in medium and late embryos.

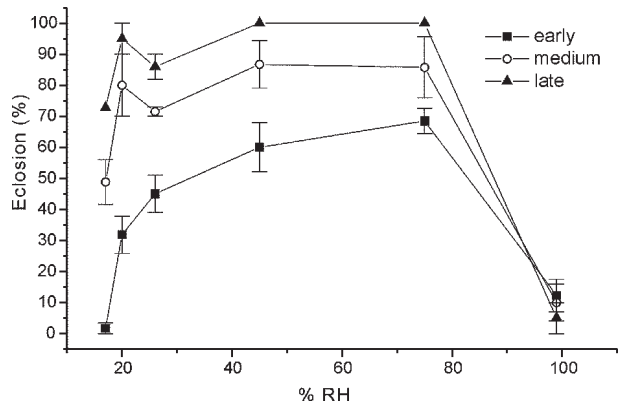


Fig. 2: effect of the relative humidity (RH) on the eclosion of early, medium, and late *Pediculus humanus capitis* eggs.

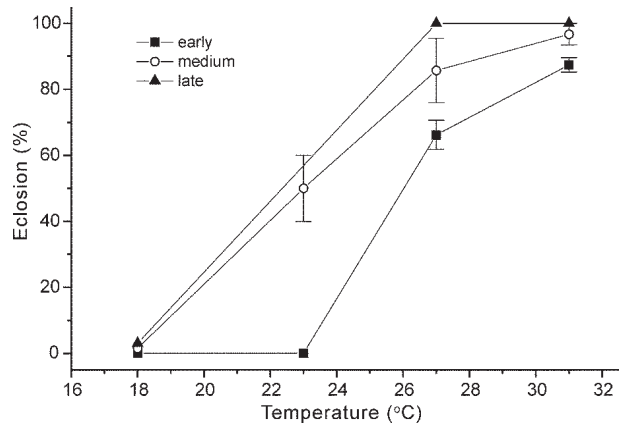


Fig. 3: effect of temperature on the eclosion of early, medium, and late *Pediculus humanus capitis* eggs incubated at different temperatures.

**Bioassay** - The treatment of eggs of each development stage of body and head lice with spinosad, caused embryo death resulting in failure to hatch. In addition it was observed that the treatment with spinosad in early and medium stage did not interrupt embryo development but it failure to hatch.

Toxicity values ( $LC_{50}$ ) to spinosad in early, medium, and late development stage, are shown in Table II. No significant difference was found between the three development stages according to the overlapped confidence limits.

Toxicity values ( $LC_{50}$ ) to spinosad and LCR between head and body lice eggs, are shown in Table III. There

was no significant difference in the susceptibility of spinosad between S-BL and GH-HL (LCR = 1).

### DISCUSSION

Developing insect eggs represent a continuous changing system that affects the toxicity of insecticides. Laboratory rearing of the developing eggs represents an important tool to select similar aged embryos for the ovidal tests. The present paper reports some changes in the morphology of human louse embryos observed through the transparent chorion at different stages of egg development. Based on these, external markers were defined for early, medium, and late eggs of head and body lice.

TABLE II

Comparative susceptible values (LC<sub>50</sub>) to spinosad in early, medium, and late embryos

Development stage	N	Slope ± SE	LC <sub>50</sub> (% w/v) (95% CL)
Early	180	1.92 ± 0.38	0.005 (0.0025-0.016)
Medium	120	0.99 ± 0.21	0.011 (0.005-0.026)
Late	130	1.15 ± 0.064	0.01 (0.003-0.03)

LC<sub>50</sub> expressed as percentage of insecticide (g) in 100 ml acetone solution.

TABLE III

Susceptibility values (LC<sub>50</sub>) to spinosad and lethal concentration ratio (LCR) in head and body louse eggs

Population	N	Slope ± SE	LC <sub>50</sub> (% w/v) (95% CL)	LCR (95% CL)
S-BL	120	0.68 ± 0.046	0.01 (0.004-0.045)	-
GH-HL	180	1.15 ± 0.064	0.01 (0.003-0.03)	1.0 (0.76-1.42)

LC<sub>50</sub> expressed as percentage of insecticide (g) in 100 ml acetone solution; LCR calculated according to Robertson and Preisler (1992).

Similar studies had been reported on the blood sucking bug *Triatoma infestans* (Klug) (Picollo de Villar et al. 1980). In this study, the changes in the external appearance of the eggs during the development allowed daily characterization of *T. infestans* embryogenesis, based mainly on ocular spots and appendages.

The development of the head louse embryo at different temperature and humidities, demonstrated that the optimal laboratory conditions for rearing *P. humanus capitis* eggs were 27-31°C and 45-75% RH. These intervals overlapped with those reported for *P. humanus humanus* (29-31°C, 40-90% RH) (Buxton 1946, Hinton 1981, Kettle 1995). Narrow intervals of environmental conditions were also reported for other louse eggs. Embryos of *Damalinia equi* (Denny) developed at 31-39°C, and embryos of *Linnognathus pedalis* (Osborne) hatched at 33-38°C (Hinton 1981). Considering humidity, embryos of *D. ovis* (Schr.) developed during water immersion with appreciable mortality after 7 day immersion, and exposure to 90% RH 24 h before hatching avoided emergence of first nymph (Hinton 1981). In *P. humanus humanus*, 70-90 % eclosion was found at 29-31°C and 40-90% RH (Buxton 1946, Hinton 1981, Kettle 1995).

Recent results from our laboratory demonstrated the high insecticide activity of spinosad against adult human lice (Mougabure Cueto et al. 2006 in press). Spinosad is an extract of fermentation of the actinomycete *Saccharopolyspora spinosa*. The spinosad alters the function of nicotinic receptors of acetylcholine and gamma-

aminobutyric acid (GABA)-gated chloride channels (Sparks et al. 2001). Based on the adult activity, it was an important goal to evaluate the effectiveness of spinosad against human louse eggs. To assess the ovicide action, we immersed eggs in acetone dilutions of spinosad. This method has not been previously reported for human louse eggs. The results showed similar susceptibility to spinosad in the 3 stages of the embryonic development of head lice. However, Picollo et al. (1976) reported that the early stage of *T. infestans* was more tolerant to organophosphate insecticides than medium and late stages, and demonstrated that the degradative enzymes present in the early stage metabolized the insecticides before of the appearance of the nervous system. On the contrary, spinosad is poorly or not readily metabolized in insect (Sparks et al. 2001). Consequently the insecticide applied to early eggs could be accumulate in the developing embryo and exert its toxic effect after the appearance of the target. This hypothesis is in accordance with the fact that head lice eggs treated in early stage almost completed its development before they died.

Toxicity values of head and body louse eggs showed similar susceptibility to spinosad, an insecticide that has not been used as pediculicide in Argentina. In agreement with these results, previous reports showed similar susceptibility for postembryonic body and head lice to insecticides not used for the control of pediculosis (Mumcuoglu et al. 1990, Hemingway et al. 1999, Lee et al. 2000, Mougabure Cueto et al. 2006. in press).

Standardized laboratory development conditions for louse eggs, identification of external markers of different stages of development, and the immersion method described in this work represent a new and alternative approach for evaluating insecticide toxicity in human louse eggs.

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