Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

J E Z S Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(2): 339-342

Received: 16-01-2017
Accepted: 17-02-2017

Ashwini M S
MVSc Scholar
Department of Veterinary Parasitology, Centre of Advanced Faculty Training, Veterinary College, KVAFSU Regional campus, Hebbal, Bengaluru, India

Puttalakshmamma GC
Associate Professor, Department of Veterinary Parasitology, Veterinary College, Hassan , Karnataka, India

Mamatha GS
Assistant Professor, Department of Veterinary Parasitology Veterinary College, Bengaluru, Karnataka, India

Chandra Naik BM
Scientists, IAH &VB Hebbal, Bengaluru, Karnataka, India

Thimmareddy PM
Professor, Department of Veterinary Parasitology Veterinary College, Bengaluru, Karnataka, India

Correspondence
Ashwini M S
MVSc Scholar
Department of Veterinary Parasitology, CAFT, KVAFSU Regional Campus, Hebbal, Bengaluru, India

In-vitro studies on biology of *Ctenocephalides orientis* fleas infesting sheep and goat

Ashwini MS, Puttalakshmamma GC, Mamatha GS, Chandra Naik BM and Thimmareddy PM

Abstract
An *in-vitro* rearing of *Ctenocephalides orientis* was carried out to study the complete biology under laboratory conditions of 25 ± 2 ºC temperature and relative humidity of 72 ± 2 percent. Holometabola type of lifecycle was observed in *C. orientis* and completed its life cycle in 31.5 ± 13.5 days from eggs to emergence of adults. The eggs were hatched in 3 ± 1 days, each larval instar took 5 ± 1 days to moult to the next stage, pre-pupal stages were formed in next 4 ± 3 days, development of pre-pupal to pupal stage completed in 2.5 ± 1.5 days and adult fleas emerged from pupal stage in next 10 ± 5 days. When the pre-pupa within the cocoon was disturbed by external factors like handling, they exited from the cocoon and developed into naked pupae. Newly emerged and fed *C. orientis* survived for a period of 6.5 ± 3.5 days and 24 ± 1 hrs without blood meal respectively.

Keywords: *In-vitro*, biology, *Ctenocephalides orientis*, sheep, goat

Introduction
Fleas are the common ectoparasites causing dermatitis both in domestic and companion animals. Heavy infestation in young animals may cause severe anaemia and death. Adult fleas parasitize the host for blood meal while remaining developmental stages occur in the host environment. Among different fleas infesting domestic animals, *Ctenocephalides orientis* is the widely distributed flea species in India on ruminants and less commonly on dog and cats [1]. *C. orientis* was earlier considered as one of the subspecies of *C. felis*. A revision of the morphology of the male reproductive organ, the phallosome and molecular studies have supported the elevation of former subspecies *C. f. orientis* to full species level of *C. orientis* [2]. However, the better understanding of flea biology is essential for implementation of effective control measures. The biology of *Ctenocephalides felis felis* and *C. canis* has been studied in detail by many authors due to their widespread distribution on cats/dogs. But there are no systematic studies conducted on biology of *C. orientis* in India except in Chennai (3). Therefore, there is paucity of information in India and the present study was carried out to understand the biology of *C. orientis* under *in-vitro* conditions.

Materials and Methods
The engorged female fleas were collected from infested sheep and goats during field outbreaks from different farms of Karnataka during the period from March 2015 to February 2016 using flea comb along with few wool fibers and flea dirt were placed in insect breeding dish with ventilated pores. Fleas were transported to the laboratory and were cleared in carbolic acid and identified morphologically as *C. orientis* (1 & 4). The number of eggs laid by single female *C. orientis* was recorded by placing ten individual female fleas in ten separate dishes and they were allowed to lay the eggs for 24 hrs. The eggs laid by the fleas were transferred into a petri plate of 5.5 cm diameter filled to a depth of 0.5 cm with a medium made of fine sand and faecal dirt of adult fleas maintained at room temperature of 25 ± 2 ^{0}C and relative humidity of 72 ± 2 percent. The eggs were examined each day for hatching and the number of days required for hatching was recorded. The larval moulting periods for next instars and to pupal stage was noted and was studied morphologically. The cocoons were removed periodically using fine painting brush and placed in an insect breeding square dish and closed with a lid. The cocoons were examined each day for emergence of adult fleas and the time interval was recorded. Emerged unfed *C. orientis* and fed *C. orientis* collected from infested sheep and goats were kept separately in an insect rearing dish and the survival period of adults without blood meal was recorded.

Results and Discussion

Morphology and morphometry of *C. orientis* developmental stages

In the present study holometabola type of lifecycle of *C. orientis* was observed in *in-vitro* conditions. On an average, *C. orientis* completed its lifecycle within a period of 31.5 ± 13.5 days from eggs to emergence of adults at 25 ± 2 º C temperature and RH of 72 ± 2 percent as shown in fig.1. Whereas, Joseph (1981) from Tamil Nadu reported that *C. f. orientis* (*C. orientis*) completed its lifecycle in 21.5 days in laboratory conditions of 32-34 ^{0}C temperature and 65-70 percent RH and the eggs were hatched in 2 days, three larval instars took 3 days each to moult into next stages, pre-pupal stage lasted for 2 days and pupal stage was completed in 8 days and adults were emerged. The variation in time taken for each development stage of *C. orientis* in compare to Joseph study could be probably due to the differences in the temperature and relative humidity as hatching of eggs, larval development, formation of pupa were dependent on environmental temperature and relative humidity. The hatching period of eggs increased when the temperature decreased. Emergence of adult fleas is also dependent on external stimuli and availability of suitable host. For every 5 ^{0}C decrease in rearing temperature in the present study, time period for emergence of all adult fleas nearly doubled [1, 4, 5, 6]. When the fleas were provided with wool fibers the fleas clinged on to the wool and moved along with the wool fibers. It reduced the damage to fleas from jumping action and duration of flea's survivability in *in-vitro* conditions was increased.

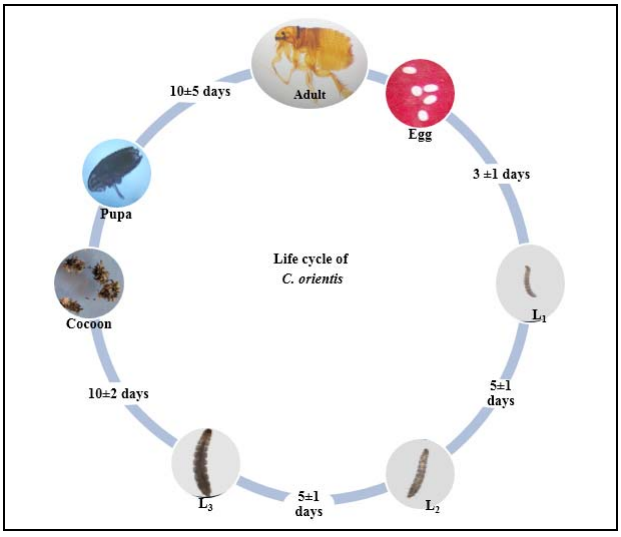

Fig 1: Life cycle of *C. orientis*

Eggs

The female *C. orientis* laid an average of 24 ± 2 eggs per day. The eggs were not clearly visible to naked eye, when viewed under stereo microscope appeared as whitish, ovoid with rounded ends, glistening and appeared like sugar granules (Fig. 2 & 3). They measured 0.5 x 0.3 mm. The freshly laid eggs were moist, sticky and slightly transparent and were adhered to the surface of jar. Later eggs dried up quickly, became opaque and darker in colour and dropped to the bottom of the jar.

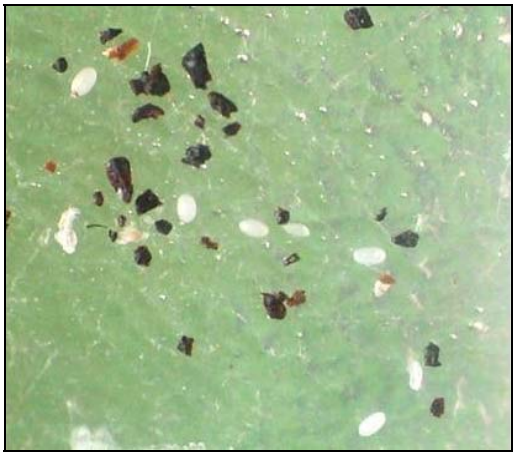

Fig 2: Eggs of *C. orientis* (40X)

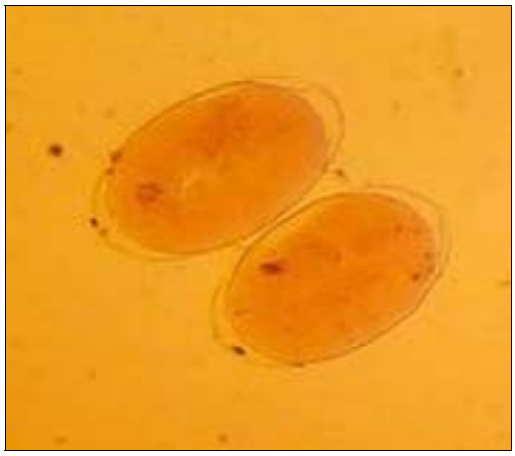

Fig 3: Eggs with developing larva (160X)

First larval instar of *C. orientis* (L_1)

The 85 ± 5 percent of the eggs were hatched into larval stage in three days. The first larval instars emerged from eggs by expanding an egg buster on the head were active, whitish in colour, eyeless, apodous, vermiform and measured about 1.75 ± 0.25 mm in length. Newly emerged larvae had a distinct head with antenna, maxillary and labial palp (Fig. 4). The body has 13 segments with no demarcation between thoracic and abdominal segments. Each segment had a circlet of backwardly-directed bristles and two hooked processes called "anal struts" on the last segment for gripping during locomotion (Fig. 5). *C. orientis* larvae were reared on faecal dirt of adults. Whereas, Joseph (3) reared in a larval medium consisted of bovine blood powder, chick mash and fine sand.

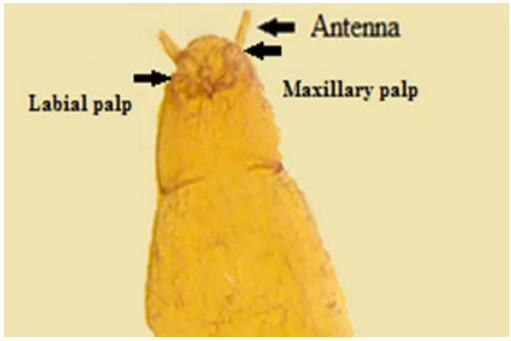

Fig 4: Ventral view of larval mouth part

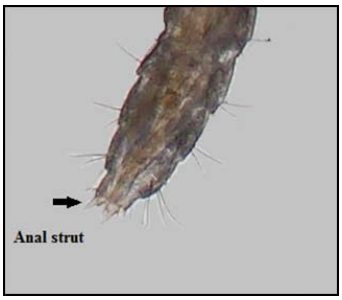

Fig 5: Posterior end (pair of anal strut)

Second larval instar of *C. orientis* (L_2)

The first larval instars were fed on flea dirt, excreted by the female *C. orientis* and moulted to subsequent instars. Second larval instars were active but had limited movements, became reddish in colour as soon as they fed on the blood-rich flea faeces and measured about 3 x 0.5 mm in size. All the three larval instars were positively geotactic (they did not crawl on the wall but remained at the bottom of the petriplate) and negatively phototactic (survived when kept in dark place). The freshly excreted flea faeces were rich in blood and quickly dried into reddish-black faecal dirt. Two types of flea dirt were found: one type was spiral-shaped and another was comma shaped (Fig. 6).

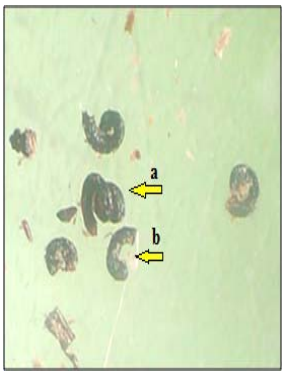

Fig 6: Faecal dirt: a. Spiral and b. Comma shape

Third larval instar of *C. orientis* (L_3)

The third larval instars measured about 4.5 ± 0. 5 mm in length and 0.5 mm in breath. They were active and reddish to whitish in colour as they begin to empty the gut contents.

Pre-pupal stage

The late third instars voided its gut contents and turned whitish in colour and were motionless in a doubled up position (U-shape) to prepare cocoon for pupation (Fig. 7).

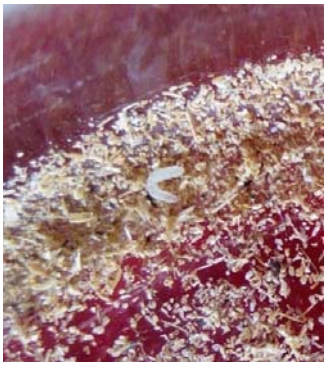

Fig 7: Gross appearance of *C. orientis* pre-pupal stage

True exarate pupa

After a short prepupal period, the mature larva transformed into true exarate pupa which is a characteristic for fleas. Then the pre-pupae spined a thin silk cocoon consisting of loose, silky and sticky threads. The cocoon was typically ovoid, sticky threads attracted the environmental debris, sand and flea faeces which provided an excellent camouflage for cocoon and measured 4 mm in length (Fig. 8). Within the cocoon the larva remained motionless and pupated. The pre-pupae needed vertical surface to successfully spin a cocoon.

Fig 8: Gross appearance of *C. orientis* cocoons

Morphological characters of naked pupa

When the pre-pupa within the cocoon was disturbed by external factors like handling, they exited from the cocoon and developed into naked pupae (Fig. 9). Initially, the pupa was whitish in colour, the legs and head were visible, later it turned reddish in colour. The colour changes started from the cranial side and progressed towards the caudal end. There was more pronounced segmentation of head, legs, thorax and abdomen. The development of eyes took place in the last stage of pupal development. The naked pupa measured about 3 mm in length and 1 mm in width. The present findings showed that the cocoon was not essential for development of pupae into adults [1, 7, 8].

Fig 9: *C orientis* naked pupa (100X).

Pre emergent and adults

The adults emerged from their cocoons showed normal movements and acrobatic jumping movement. The adult *C. orientis* had both genal and pronotal combs. The anterior most genal spine was shorter than subsequent spine. The length of head was twice as long as height and the frons were elongate and broadly rounded at anterior end. Metepisternum or lateral

metanotal area (LMA) had two bristles. Females had a row of one to 12 short spiniform bristles behind the antennal groove. Dorsal margin of hind tibia had seven notches with stout and single bristles on third and sixth notches. Chaetotaxy formula of metatibial bristles was 2-2-1-2-2-1-3. In males, the manubrium of the clasper was widened at the apex.

Morphological differentiation of male and female fleas

Adult male and female fleas were identified based on the size, shape of the abdomen and genital organ (spermatheca in females and complex copulatory apparatus in males). Female fleas were bigger in size (2.75 ± 0.25 x 1.25 ± 0.25) compared to males (2.25 ± 0.25 x 1 ± 0.25 mm). Both dorsal and ventral surface of the abdomen was convex in females whereas, dorsal surface was more or less flat and the ventral surface was greatly curved in males. There was a C shaped spermatheca on ninth abdominal segment of females (Fig. 10). In males, paired manubrium, articulating clasper and L shaped clasping organ were clearly defined (Fig. 11).

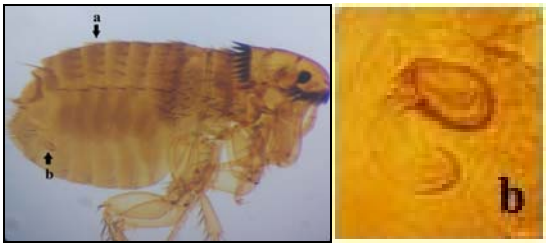

Fig 10: ***C. orientis*** female: a. Shape of the abdomen and b. Spermatheca

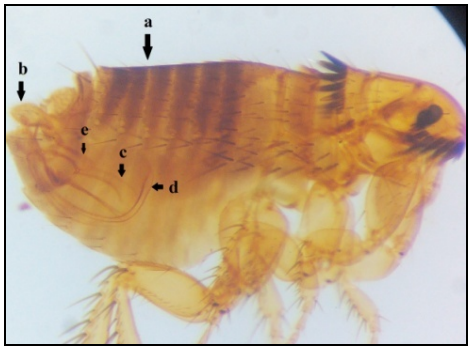

Fig 11: ***C. orientis*** male: a. Shape of the abdomen; b. Movable process of clasper; c. Penis plate; d. Spring of penis and e. Manubrium

The present findings of morphology and morphometry of *C. orientis* flea developmental stages were in agreement with Taylor, Bitam & Dobler [1, 5, 9].

In the present study, unfed *C. orientis* survived for an average period of 6.5 ± 3.5 days without feeding and fed *C. orientis* collected from the host survived for only 24 ± 1 hrs without blood meal compare to the longevity of unfed fleas because the survivability of unfed fleas depends upon the environmental humidity and temperature during cocoon formation [1, 8] and adult fed fleas required to feed every 12 hrs in order to survive [10].

To conclude, *C. orientis* completed its lifecycle in an average period of 31.5 ± 13.5 days at 25 ± 2 ^{0}C temperature and RH of 72 ± 2 percent. Adult *C. orientis* parasitize the host while the developmental stages occur in the environment. Therefore, the control measures should be aimed at both host as well as the environment every month for a period of six months to kill all the stages of fleas and also to prevent re-infestation.

Acknowledgements

Authors are thankful to CAFT (Centre of Advanced Faculty Training), Department of Veterinary Parasitology, Veterinary College, Bengaluru for providing necessary facilities for conducting this research.

References

1. Taylor MA, Coop RL, Wall RL. Veterinary Parasitology. Eds 3, Blackwell, 2007, 715-725.
2. Lawrence AL, Brown GK, Peters B, Spielman DS, Morin-adeline V, Slapeta J. High phylogenetic diversity of the cat flea (*Ctenocephalides felis)* at two mitochondrial DNA markers. Medical and Veterinary Entomology. 2014; 28:330-336.
3. Joseph SA. Studies on the bionomics of *Ctenocephalides felis orientis.* Cherion. 1981; 10(6):275-280.
4. Marquardt WC, Demaree RS, Grieve RB. Parasitology and Vector Biology. Edn 2. 2000, 569-582.
5. Bitam I, Dittmar K, Parola P, Whiting MF, Raoult D. Fleas and flea-borne diseases. Inter. Journal of Infectious Diseases. 2010; 14:667-676.
6. Devi S, Deka DK, Neog R, Islam S, Upadhyaya TN, Das M. Studies on the life cycle of *Ctenocephalides felis felis* vis-a-vis development of cysticercoids of *Dipylidium caninum.* Journal Veterinary Parasitology. 2011; 25(2):177-178.
7. Kettle DS. Medical and Veterinary Entomology. Edn 2. Chapter 1995; 17:23-343.
8. Rust MK, Dryden MW. The biology, ecology and management of the cat flea. Annual Review of Entomology. 1997; 42:451-73.
9. Dobler G, Pfeffer M. Fleas as parasites of the family canidae. Parasites and Vectors. 2011; 4:139.
10. Hinkle NC, Koehler PG, William HK. Hematophagous strategies of the cat flea (Siphonaptera: Pulicidae). Florida Entomologist. 1991; 74(3):377-385.