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Blood feeding of *Ornithodoros turicata* larvae using an artificial membrane system

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Abstract

An artificial membrane system was adapted to feed *Ornithodoros turicata* (Ixodida: Argasidae) larvae from a laboratory colony using defibrinated swine blood. Aspects related to larval feeding and moulting to the first nymphal instar were evaluated. A total of 55.6% of all larvae exposed to the artificial membrane in two experimental groups fed to repletion and 98.0% of all fed larvae moulted. Mortality rates of first instar nymphs differed significantly depending on the sorting tools used to handle engorged larvae ($\chi^2 = 35.578$, $P < 0.0001$): engorged larvae handled with featherweight forceps showed significantly higher mortality (odds ratio = 4.441) than those handled with a camel-hair brush. Differences in the physical properties of the forceps and camel-hair brush may affect the viability of fragile soft tick larvae even when care and the same technique are used to sort them during experimental manipulations. The current results represent those of the first study to quantify successful feeding to repletion, moulting and post-moulting mortality rates in *O. turicata* larvae using an artificial membrane feeding system. Applications of the artificial membrane feeding system to fill gaps in current knowledge of soft tick biology and the study of soft tick–pathogen interactions are discussed.

Keywords

Artificial feeding; soft tick; *Ornithodoros*

Blood-feeding techniques have been applied in the laboratory to a wide range of haematophagous arthropods since the pioneering work of Hindle & Merriman (1912) and Rodhain et al. (1912) to study vector biology, vector–pathogen interactions and colony

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maintenance, and to test compounds for vector control. Laboratory blood-feeding techniques using artificial membranes offer relative simplicity and the potential to reduce or avoid live animal use (Butler et al., 1984). However, longterm laboratory maintenance of haematophagous arthropods may affect fitness (Costa-da-Silva et al., 2014). This is of particular relevance in ticks (Acari), in which the tick-host interplay can affect reproduction and fecundity (Allan, 2014).

Different artificial feeding techniques, such as those using membranes and capillary tubes, have been applied to feed several species of Argasidae in the laboratory (Tarshis, 1958; Hokama et al., 1987; Schwan et al., 1991; Ben-Yakir & Galun, 1993; Abbassy et al., 1994). However, successful artificial membrane feeding of *Ornithodoros turicata* (Dugès, 1876) has been documented rarely. Butler et al. (1984) reported successful artificial membrane feeding in all stages of *O. turicata*, but did not quantify rates of feeding to repletion, moulting and survival to subsequent stages. Zheng et al. (2015) recently characterized biological and physiological responses of late instar nymphs and adult *O. turicata* blood-fed through an artificial membrane system equipped for respirometry and electrophysiology recordings.

Ornithodoros turicata is a recognized vector of *Borrelia turicatae* (Brumpt, 1933), a causative agent of human tick-borne relapsing fever in the U.S.A. (Davis, 1943). In addition, this species has been experimentally infected with African swine fever virus (ASFV), of which it is considered a potential vector (Hess et al., 1987). Efficient and reliable artificial feeding techniques that support all life stages of *O. turicata* are required to enhance current understanding of the biology, vector–host–pathogen interactions and colony maintenance of this soft tick species of veterinary and medical importance. The present report describes a technique to artificially feed *O. turicata* larvae and notes observations on moulting success to first instar nymphs.

Ornithodoros turicata larvae aged 3 months were obtained from a colony maintained at the Tick Research Laboratory, Texas A&M AgriLife Research (College Station, TX, U.S.A.). The colony originated from specimens collected in a natural cavern in Travis County, Texas in 1992, and was maintained under an LD 14 : 10h photoperiod, $25 \pm 3^{\circ}\text{C}$ and 80–85% relative humidity (RH) using young cockerels (*Gallus gallus*) as bloodmeal hosts according to procedures approved by the Institutional Animal Care and Use Committee of Texas A&M University (AUP no. 2014-255).

A modified blood-feeding apparatus was adapted for this study based on designs reported by Butler et al. (1984), Schwan et al. (1991) and Zheng et al. (2015). Feeding chambers were made from 50-mL conical polypropylene screwcap centrifuge tubes (Thermo Fisher Scientific, Inc., Waltham, MA, U.S.A.) cut at the bottom to lengths of 4.5 cm. A rectangular strip of Parafilm M® (Bemis Co., Inc., Oshkosh, WI, U.S.A.) was used as a membrane by stretching it to maximum capacity over the bottom (the cut side) of each chamber. One half of a 100-mm glass Petri dish (Kimble Science and Research Products LCC, Rockwood, TN, U.S.A.) was filled with 20 mL of blood and suspended in a water bath (Thermo Fisher Scientific Co., Fair Lawn, NJ, U.S.A.) to maintain the blood temperature at $34 \pm 2^{\circ}\text{C}$ (Fig. 1). Commercially available, mechanically defibrinated swine blood (Rockland Immunochemical, Limerick, PA, U.S.A.) was used as a bloodmeal source.

Two experimental groups of *O. turicata* larvae were evaluated for successful blood feeding in this artificial system. Larvae were sorted over the course of pre-feeding, feeding and post-feeding transitions using narrow-tipped featherweight forceps (BioQuip Products, Inc., Rancho Domingo, CA, U.S.A.) in Group 1 larvae and small camel-hair brushes in Group 2 larvae. In Group 1, 60 *O. turicata* larvae were placed in each of 10 feeding chambers (total: 600 larvae) with the featherweight forceps and allowed to feed for 120 min. In Group 2, 60 *O. turicata* larvae were placed in each of five feeding chambers (total: 300 larvae) with the small camel-hair brush and allowed to feed for 120 min. All engorged larvae from each experimental group were collected upon completion of the feeding period, transferred to vials using the respective sorting tool for the group and kept under standard colony maintenance conditions to facilitate the daily observation of the moulting progress.

JMP Pro 12 statistical software (SAS Institute, Inc., Cary, NC, U.S.A.) was used to conduct chi-squared tests for homogeneity of proportion and to determine odds ratios (ORs) to compare morality rates between experimental groups.

Table 1 summarizes the responses of larvae in the two experimental groups fed on the artificial membrane. Mortality rates in first instar nymphs differed significantly depending on the sorting tool used to handle engorged larvae ($\chi^2 = 35.578$, $P < 0.0001$): engorged larvae handled with featherweight forceps had a significantly higher mortality rate (OR = 4.441) than those handled with the camel-hair brush. In the group sorted with forceps, 345 of 600 larvae (57.5%) successfully fed to repletion through the membrane. The majority of fed larvae ($n = 339$, 98.3%) subsequently moulted to first instar nymphs between days 14 and 26 post-feeding.

A mortality rate of 35.4% (122 of the 345 successfully fed larvae) was observed in the group sorted with forceps; 95.0% of these deaths occurred during or shortly after ecdysis. In fed larvae, the rate of successful transition from larva to first instar nymph was 64.6%.

In the group sorted by camel-hair brush, 155 of 300 larvae (51.7%) successfully fed to repletion and 151 of 155 (97.4%) moulted to first instar nymphs between days 14 and 20 post-feeding. A mortality rate of 11.0% (17 of 155 successfully fed larvae) was observed, with all mortality occurring shortly after ecdysis. The rate of successful transition from fed larva to first instar nymph was 89.0%. The comparative overall yields of first instar nymphs (total surviving first instar nymphs of total unfed larvae) were 37.2 and 46.0% in *O. turicata* immatures sorted with forceps and camel-hair brushes, respectively. Finally, two larval feeding behaviours were observed to include feeding in cluster formation and feeding individually (Fig. 2).

The current results represent those of the first study to quantify successful feeding to repletion, moulting and post-moulting mortality in *O. turicata* larvae, and their transition to first instar nymphs using an artificial membrane feeding system. Remarkably, different effects on post-moult mortality were observed in association with the tools used to sort larvae. Mortality associated with the use of camel-hair brushes was approximately one-third of that observed in larvae sorted with featherweight forceps. One plausible explanation may

be that sorting engorged larvae with featherweight forceps caused undefined damage (e.g. midgut rupture) that led to post-moult mortality.

The feeding system used in this study had one unforeseen outcome. Prior attempts to feed *Ornithodoros concanensis* (Cooley & Kohls, 1941) late instar nymphs in the present group's laboratory (Kirch et al., 1991), which involved the use of a Parafilm M® membrane over screen-topped open chambers, resulted in the formation of blood pools at the site of tick mouth-part penetration during feeding. The authors were concerned that the formation of blood pools at the surface of the membrane might impede the successful feeding and moulting of the smaller *O. turicata* larvae. However, no blood pool formation was noted at larval attachment sites with the feeding system used in this study. One explanation for this may refer to differences in the tick stages used, size of chelicerae and hypostome structure between the two species. Another explanation may reflect a difference in feeding chamber construction. The *O. concanensis* nymph experiments were conducted using screen-topped chambers, whereas the chambers used in the present system used screwcap lids that were likely to provide a more airtight seal. *Ornithodoros turicata* larvae in this study were loaded into the feeding chamber at room temperature and subsequently exposed to a higher temperature over the water bath. This may have expanded the air inside the sealed feeding chamber, creating a positively pressured system that prevented blood from moving into the feeding chamber once larvae penetrated the membrane with their mouthparts.

The present results indicate that *O. turicata* could potentially complete its lifecycle in the laboratory using an *in vitro* blood-feeding system, but experiments to prove successful nymph and adult feeding, as well as egg production, remain to be completed. This study complements the work of Zheng et al. (2015) demonstrating that nymphs and adults can feed to repletion in a system using an artificial membrane. Additional studies are planned to evaluate mating and fecundity in *O. turicata* feeding in the artificial membrane system. The current results validate the observation made by Butler et al. (1984) that *O. turicata* larvae can feed through an artificial membrane. Moreover, the quantification of feeding to repletion, moulting and post-moulting mortality rates facilitates the estimation of the overall yield of first instar nymphs from groups of larvae fed using an artificial feeding system.

Artificial membrane feeding systems such as that described here may provide several advantages over the feeding of ticks on live animals. In addition to reducing the number of animals used to feed each life stage, an *in vitro* blood-feeding system with an artificial membrane could replace the need for animals in order to maintain a laboratory colony and allow relevant experiments to be conducted in a more economic manner. Importantly, given that *O. turicata* is a non-selective feeder, an artificial membrane feeding system would facilitate host blood source competency and fecundity studies. Additionally, the artificial feeding system could be applied in vector efficiency studies involving host blood with various anthropogenic conditions such as immunization, chemoprophylaxis and systemic pesticides (Butler et al., 1984).

Efficient artificial feeding techniques enable the study of tick-pathogen interactions, argasid physiology and feeding behaviour under controlled conditions (Butler et al., 1984). For example, a specific inoculum required for vector colonization could be quantified by

infecting *Ornithodoros* ticks with relapsing fever *Borrelia* or ASFV, which currently requires live mammalian hosts. Moreover, with the emergence of new genetic information for relapsing fever spirochetes, the intricacies of midgut and salivary gland colonization can be further defined using an artificial feeding system. Finally, the ability to use various blood sources in the artificial membrane feeding system could provide the flexibility needed to rear other *Ornithodoros* species in the laboratory.

The survival and fitness of *Ornithodoros* spp. fed on artificial membrane systems remain to be fully elucidated. To the best of the current authors' knowledge, data on the performance of each life stage exist only for *Ornithodoros moubata moubata* (Murray, 1877) (Schwan et al., 1991). However, this argasid species does not feed in the larval stage. Because field population and ecological data for argasids are scarce, research on soft tick survival and fitness using laboratory colonies such as that reported here can provide important reference information to advance current knowledge of this understudied group of Acari.

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References

- Abbassy MM, Stein KJ & Osman M (1994) New artificial feeding technique for experimental infection of Argas ticks (Acari: Argasidae). *Journal of Medical Entomology*, 31, 202–205. [PubMed: 8189410]
- Allan SA (2014) Tick rearing and *in vitro* feeding *Biology of Ticks*, Vol. II (ed. by Sonenshine DE & Roe RM), pp. 443–473. Oxford University Press, New York, NY.
- Ben-Yakir D & Galun R (1993) Comparative study of two argasid tick species: feeding response to phagostimulants. *Israel Journal of Zoology*, 39, 169–176.
- Butler JF, Hess WR, Endris RG & Holscher KH (1984) *In vitro* feeding of *Ornithodoros* ticks for rearing and assessment of disease transmission *Acarology VI*, Vol. 2 (ed. by Griffiths DA & Bowman CE), pp. 1075–1081. Ellis Horwood, Chichester.
- Costa-da-Silva AL, Carvalho DO, Kojin BB & Capurro ML (2014) Implementation of the artificial feeders in hematophagous arthropod research cooperates to the vertebrate animal use replacement, reduction and refinement (3Rs) principle. *Journal of Clinical Research & Bioethics*, 5, 1–3. [PubMed: 25590017]
- Davis GE (1943) Relapsing fever: the tick *Ornithodoros turicata* as a spirochetal reservoir. *Public Health Reports*, 58, 839–842.
- Hess WR, Endris RG, Haslett TM, Monahan MJ & McCoy JP (1987) Potential arthropod vectors of African swine fever virus in North America and the Caribbean basin. *Veterinary Parasitology*, 26, 145–155. [PubMed: 3326244]
- Hindle E & Merriman G (1912) The sensory perceptions of *Argas persicus* (Oken). *Parasitology*, 5, 203–216.
- Hokama Y, Lane RS & Howarth JA (1987) Maintenance of adult and nymphal *Ornithodoros coriaceus* (Acari: Argasidae) by artificial feeding through a parafilm membrane. *Journal of Medical Entomology*, 24, 319–323.
- Kirch HJ, Teel PD, Kloft WJ & Deloach JR (1991) Artificial feeding of *Ornithodoros concanensis* (Acari: Argasidae) nymphs on bovine blood and morphological changes in erythrocytes

undergoing hemolysis in the tick midgut. *Journal of Medical Entomology*, 28, 450–455. [PubMed: 1875374]

Rodhain J, Pons C, Vandenbranden J & Bequaert J (1912) Contribution towards the transmission mechanism of trypanosomes by glossines. *Archiv für Schiffs- und Tropen-Hygiene*, 16, 732–739.

Schwan EV, Hutton D, Shields KJB & Townson S (1991) Artificial feeding and successful reproduction in *Ornithodoros moubata moubata* (Murray, 1877) (Acarina: Argasidae). *Experimental & Applied Acarology*, 13, 107–115. [PubMed: 1786742]

Tarshis IB (1958) A preliminary study on feeding *Ornithodoros savignyi* (Audouin) on human blood through animal-derived membranes (Acarina: Argasidae). *Annals of the Entomological Society of America*, 51, 294–299.

Zheng H, Li AY, Teel PD, Pérez de León AA, Seshu J & Liu J (2015) Biological and physiological characterization of *in vitro* blood feeding in nymph and adult stages of *Ornithodoros turicata* (Acari: Argasidae). *Journal of Insect Physiology*, 75, 73–79. [PubMed: 25783956]

Fig. 1. Artificial blood-feeding apparatus for *Ornithodoros turicata* larvae. (A) Feeding chambers were made from 50-mL conical polypropylene screwcap centrifuge tubes cut at the bottom to lengths of 4.5 cm and sealed with stretched Parafilm M® membrane. (B) Feeding chambers were placed on one half of a 100-mm glass Petri dish filled with 20 mL of defibrinated blood and (C) placed on top of a test tube rack in a (D) water bath to maintain the blood at a temperature of $34 \pm 2^\circ\text{C}$.

Fig. 2.

(A) Feeding behaviours in *Ornithodoros turicata* larvae included feeding in cluster formation and feeding individually. (B) A total of 89.0% of larvae that fed to repletion made a successful transition to first instar nymphs.

Feeding, moulting and post-feeding survival rates of *Ornithodoros turicata* larvae in an artificial feeding system according to the tool used to sort the ticks.

Table 1.

Feeding group (sorting tool) *	Larvae allowed to feed, n	Larvae fed, n (%)	Larvae moulted [†] , n (%)	Pre-moult period, days, range	Mortality in engorged and newly moulted larvae [*] , n (%)	Surviving first instar nymphs [*] n (%)
Featherweight forceps	600	345 (57.5%)	339 (98.3%)	14–26	122 (35.4%)	223 (64.6%)
Camel-hair brush	300	155 (51.7%)	151 (97.4%)	14–20	17 (11.0%)	138 (89.0%)

* Used for likelihood analysis (chi-squared test) and odds ratio determination.

[†] Includes both dead and surviving larvae.