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Development cycle of *Ornithodoros peruvianus* Kohls, Clifford & Jones (Ixodoidea: Argasidae) under laboratory conditions

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**ABSTRACT**

The objective of this study was to describe the developmental biology of the tick *Ornithodoros peruvianus* Kohls, Clifford & Jones under laboratory conditions. A total of 174 live specimens of *O. peruvianus* at different stages were collected from caves located on Pan de Azúcar Island (26°09’S, 70°41’W) and Tal Tal (25°15’S, 70°15’W). In the laboratory, ticks were fed on rabbits (*Oryctolagus cuniculus*) and kept in acrylic tubes under controlled conditions of humidity and temperature (average of 75% ± 5 and 23 ± 1°C, respectively), and a 12 hour photoperiod. The minimum period between stages were: oviposition to egg hatching: 9 days; larva to nymph one: 44 days (44-56); nymph one to nymph two: 10 days (10-20); nymph two to male: 66 days (66-84); nymph two to nymph three: 68 days, and nymph three to female: 42 days. Oviposition occurred in 24 females, with an average of 70 (15-133) eggs per female after the first bloodmeal. The total time to complete the cycle under laboratory conditions was 185 days on average (SD = 17.43). *O. peruvianus* may require three nymphal stages to molt into an adult female and only two to molt into an adult male. The selected environmental conditions, host and photoperiod are important factors that must now be considered to better understand the factors that affect developmental times of this tick.

**Keywords** Soft tick, life cycle, nymph, larvae, Chile

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**Introduction**

The biology of ticks *in situ* is complex, such that the development of *ex situ* biological cycles may be useful in elucidating their natural history. The genus *Ornithodoros* Koch, 1937 (Acari: Argasidae) includes 116 described species worldwide, the majority of the 200 species that make up the family Argasidae (Guglielmone et al. 2010, Nava et al. 2010, Dantas-Torres et al. 2012, Nava et al. 2013, Venzal et al. 2013, Barros-Battesti et al. 2015, Labruna et al. 2016, Muñoz-Leal et al. 2016). Only five species are known from Chile, which are *O. amblus* Chamberlin, 1920, *O. spheniscus* Hoogstraal, Wassef, Hays & Keirans, 1985 (González-Acuña et al. 2008), *O. peruvianus* Kohls, Clifford & Jones, 1969 (Kohls et al. 1969), *O. microlophi*
Venzal, Nava & González-Acuña, 2013 (Venzal et al. 2013) and O. atacamensis Muñoz-Leal, Venzal & González-Acuña, 2016 (Muñoz-Leal et al. 2016). O. peruvianus was described for the first time in 1969 by Kohls, Clifford & Jones in the bat Desmodus rotundus Geoffroy Saint Hilaire, 1810 from Perú. Years later Venzal et al. (2012) described this species parasitizing D. rotundus in Chile. Little is known about the general biology of this tick in Chile or elsewhere. We therefore conducted the present study to obtain information on the developmental biology of O. peruvianus under laboratory conditions.

Materials and methods

From April 2010 to March 2012, 174 specimens (36 females, 36 males and 102 nymphs) of O. peruvianus were collected from bat colonies in caves on Pan de Azucar Island (26°09’S, 70°41’W), Atacama region and Tal Tal (25°24’02’S, 70°30’51”W), Antofagasta Region. At the time of tick collection, the temperature and relative humidity (RH) were measured inside the cave and these data were then used to select the environmental conditions in which the Ornithodorous biological cycle would be developed. The tick species was determined using published taxonomic descriptions (Nava et al. 2010). Four rabbits Oryctolagus cuniculus (Linnaeus, 1758) were used as hosts for all parasitic stages of O. peruvianus. In each rabbit, the right and left flanks were shaved; two acrylic containers (3 cm in diameter and 2 cm long), open at the end in contact with the skin and closed with a perforated cap at the other end, were attached to each shaved zone with non toxic adhesive tape (Medipore H, 3M). In each container, a female, a male and a nymph were deposited at the same time. Newly hatched larvae were observed until mouth parts had hardened and were then placed to feed in the acrylic containers in groups of 20 individuals. Feeding time was measured from the moment the tick attached to the host until it fell off.

Rabbits were checked daily for naturally detached engorged ticks, which were placed in a plastic tube with small holes in the cap; adults that were placed in couples. In the laboratory, the ticks were held in an incubator (EARLY WH71, Seoul, South Korea) at 23 ± 1 °C and 75 ± 5% RH and a 12 hour photoperiod. Ticks were observed daily with a magnifying glass (ZEISS Stemi DV4, Germany, Jena) to determine preoviposition period (period between the last feeding and the start of egg laying), oviposition length (days to complete egg laying), daily number of eggs laid, and the incubation time (day from oviposition to egg hatching). Hatching rate was also determined by comparing the number of eggs laid with the number of hatched larvae. The larvae, once fed, were placed individually in plastic tubes and observed daily to record molt time and the success rate of these molts (number of viable ticks post-molt). After each molt, nymphs were fed and observed until reaching the adult stage. The total life cycle was calculated from the day of oviposition (day 1) until the last nymphal stage molted into the adult stage.

This study was approved by the Comité de Ética, Facultad de Ciencias Veterinarias, Universidad de Concepción (CE-03-2009).

Results

The life cycle of O. peruvianus at 23 ± 1 °C and 75 ± 5% RH was completed on average in 185 days (SD=17.43). The cycle included a single larval stage, 2 – 3 nymphal instars and one adult stage, female or male. Adult males originated from nymph two and females from nymph three. Only 24 females laid eggs out of the 36 collected from the field. The average number of eggs laid was 70.1 (SD=28.34) during the first gonadotrophic cycle of the females (Table 1). Observed preoviposition, oviposition and incubation periods showed a fairly wide range. Egg eclosion was 59.05%. After hatching all larvae had a maturity period of 10 days by which time mouthparts were found suitable for feeding. Six specimens that emerged from eggs reached the
Table 1 Biological parameters of the life cycle of *Ornithodoros peruvianus* fed on rabbits and maintained at 23°C, 75 ± 5% RH at 12: 12 h L:D photoperiod.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>N</th>
<th>Average time (days)</th>
<th>Standard deviation</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>N° of eggs laid per female</td>
<td>24</td>
<td>70.1</td>
<td>28.34</td>
<td>15-133</td>
</tr>
<tr>
<td>% hatched</td>
<td>24</td>
<td>59.05</td>
<td>25.35</td>
<td>9.1-133</td>
</tr>
<tr>
<td>Preoviposition period</td>
<td>24</td>
<td>37.5</td>
<td>32.81</td>
<td>9-133</td>
</tr>
<tr>
<td>Oviposition period</td>
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<td>14.7</td>
<td>13.25</td>
<td>3-65</td>
</tr>
<tr>
<td>Egg incubation period</td>
<td>24</td>
<td>9.8</td>
<td>4.50</td>
<td>5-22</td>
</tr>
<tr>
<td>Larva to Nymph I</td>
<td>6</td>
<td>47.3</td>
<td>4.81</td>
<td>44-56</td>
</tr>
<tr>
<td>Nymph I to Nymph II</td>
<td>6</td>
<td>14.83</td>
<td>4.03</td>
<td>10-20</td>
</tr>
<tr>
<td>Nymph II to Nymph III</td>
<td>1</td>
<td>68</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nymph II to Male</td>
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<td>74.6</td>
<td>7.60</td>
<td>66-84</td>
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<tr>
<td>Nymph III to Female</td>
<td>1</td>
<td>42</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total time 1</td>
<td>6</td>
<td>185</td>
<td>17.43</td>
<td>167-216</td>
</tr>
</tbody>
</table>

1 from oviposition to adult molt

adult stage. Of these, five molted into adult males and one into an adult female. All measured biological parameters of the cycle of *O. peruvianus* are shown in Table 1.

Nymphs and adults had shorter feeding times (minutes) than larvae (at least 7 days). An average of 17.6 minutes was recorded with a minimum of 5.1 min and a maximum of 35 min in nymphs. All nymphs (I, II and III) fed once before molting to the next stage. The 36 females of *O. peruvianus* that fed on rabbits presented a mean feeding time of 32.9 mins (range 10 min to 126 min), while the 36 males fed 23.3 minutes on average (range of 6 min to 53 min). During and after feeding, ticks of both sexes produced large amounts of coxal fluid.

**Discussion**

No previous study has focused specifically on the life cycle biology of *O. peruvianus* under laboratory conditions. However, several studies have examined this in related species of the genus *Ornithodoros* that exploit bat hosts. The present work described the presence of a larva, three nymphal instars and adults in the life cycle of *O. peruvianus*. In *O. kelleyi* Cooley and Khols, 1941, two to four nymphal instars have been observed before adult molt (Sonenshine and Anastos 1960). The life cycle of *O. mimon* Kohls, Clifford & Jones, 1969 is more similar to *O. peruvianus* with three nymphal instars before the molt into the adult stage (Landulfo et al. 2012). However, in *O. mimon* it was observed that nymph II can molt into either an adult male or adult female (Landulfo et al. 2012), whereas in our study only nymph III molted into a female. More generally, *O. peruvianus* has a wider range (167-216 days) and seems to require more time to complete the cycle than other ticks of the genus *Ornithodoros*. For example, Landulfo et al. (2012) reported that *O. mimon* had a life cycle lasting between 146-175 days under a temperature of 27 ± 1°C and 90 ± 10% relative humidity (RH). *O. rostratus* Aragao, 1911 was decrised to have a life cycle lasting between 66 to 136 days under a temperature of 27 ± 1°C and 80 ± 10 % (Ribeiro et al. 2013), whereas *O. kelleyi* completed its cycle between 54-258 days at 30°C and 77% RH (Sonenshine and Anastos 1960). In the same study of *O. mimon*, Landulfo et al. (2012) found that engorged larvae took 6.8 ± 0.5 days to reach the first nymphal instar and first stage nymphs took 9-14 days to molt into second stage nymphs. The step from nymph II to nymph III was obtained in 10-14 days. Finally, nymph III took 14-17 days to molt into adult males and 12-19 days to molt into adult females. These inter-molt times are lower than those recorded in the present study.

Compared to the previous species, the cycle of *O. peruvianus* was studied at a lower temperature (23°C). The duration of the biological cycle is known to decrease with increasing
temperature. For example, in *Otobius megnini* (Dugès, 1844), high temperatures accelerated egg hatching and incubation times (Diyes and Rajakaruna 2017). In *O. erraticus* (Lucas, 1849), El Shoura (1987a) showed that the egg incubation period increased when incubation temperature decreased, observing that the longest incubation period occurred at 22 °C. The effect of different RH was less pronounced. In the same way, Endris et al. (1991) described that decreasing temperature increased the incubation time, the development time from the first to the fourth nymphal instar and the mean duration of the gonotrophic cycle in *O. puertoricensis*.

The photoperiod may also influence the feeding time, oviposition and development of the ticks. For example, in hard ticks *Haemaphysalis longicornis*, *Ixodes persiculatus*, *Rhipicephalus decoloratus*, *R. geigyi*, *R. sanguineus* and *Haemaphysalis leachi leachi*, an increase in the frequency of oviposition has been observed in periods of scotophase (Fujisaki et al. 1973, Amoo 1984, Adejinmi and Akinboade 2012). However in other ticks no effect has been observed (eg. *Rhipicephalus annulatus*, Ouhelli et al. 1982). In soft ticks, Adeyeye and Philips (1996) evaluated the effect of photoperiod on feeding, development and quiescent behavior of *Ornithodoros turicata*. They observed that ticks reared in continuous darkness developed more slowly than those reared under short-day or long-day conditions. However, the egg-hatch success was significantly higher for ticks reared in continuous darkness, and the pre-oviposition period was significantly shorter than for ticks reared in long-day conditions. In our study, we selected a 12/12 photoperiod; these conditions could have influenced the development, number of eggs and hatching success.

Another factor that could influence the length of the biological cycle is the host on which ticks feed. The rabbit is not a natural host for *O. peruvianus*. This species was chosen because it is a good experimental animal, easily handled and nonlaborious to maintain (Faccini et al. 2006). Rabbits have been used to examine the biological cycles of other *Ornithodoros* species (Landulfo et al. 2012, Ribeiro et al. 2013) and other Argasidae (ex. *Otobius megnini*) (Diyes and Rajakaruna 2017). These studies indicate that rabbits were suitable hosts for describing the development cycle in the laboratory. However, there are differences according to the host used. For example in *O. mimon*, ticks fed on gerbils and rabbits had different molting and feeding times (Landulfo et al. 2012). For *O. kelleyi*, it was possible to feed larvae on non-natural hosts, but nymphs and adults were only able to feed natural hosts (bats). Although feeding time may vary according to the host used, this variation remains relatively narrow among mammal hosts. For example, the average feeding time for *O. puertoricensis* on rats, mice and guinea pigs varied between 3.1 and 6.8 days (Davis 1955, Endris et al. 1991, Fox 1947). This difference becomes much more pronounced when comparing mammalian and reptilian hosts (Venzal and Estrada-Peña 2006).

The preoviposition period in *O. peruvianus* described in this study showed values much closer to the species *O. kelleyi* (Sonenshine and Anastos 1960) than to *O. mimon* (Landulfo et al. 2012). Indeed, the average for *O. peruvianus* proved to be almost twice that obtained for *O. mimon* (Sonenshine and Anastos 1960, Landulfo et al. 2012). Landulfo et al. (2012) reported that *O. mimon* laid an average of 137 eggs with a minimum of 84 and a maximum of 226 eggs, and had a hatching success rate of 77% on average (range of 60 to 92%). Both egg numbers and hatching success was lower for *O. peruvianus*. However, in our study, only eggs from the first gonotrophic cycle were recorded. The first gonotrophic cycle may have a smaller number of eggs per female because females are not yet fully mature, and part of the blood ingested in the first feeding may be used for development instead of egg production (Schumaker and Barros 1995).

The feeding time of larvae was similar to that recorded in other species of bat ticks. According to Ribeiro et al. (2013), three larval classes can be defined within the genus *Ornithodoros* according to their feeding pattern: group 1 = larvae to nymph I without feeding, group 2 = larvae feed for a few minutes, and group 3 = larvae feed slowly many days. *O. peruvianus* falls within the third group along with other species which parasitize birds and bats (Sonenshine and Anastos, 1960; Schumaker et al., 1997; Landulfo et al., 2012). In our study, larvae were able to feed 10 days post-hatching. Similar values were observed by Landulfo et
In this study, we observed that during and after feeding, ticks of both sexes produced large amounts of coxal fluid, which is consistent with descriptions by various authors for different species of the genus *Ornithodoros* (Sonenshine and Anastos 1966, Vargas 1984, Beck et al. 1986, El Shoura 1987b, Endris et al. 1991, Martins et al. 2011, Landulfo et al. 2012, Ribeiro et al. 2013). It has been suggested that the coxal fluid produced by the females has sex pheromones that attract the male for copulation (El Shoura 1987b), but no test of this hypothesis has yet been made.

Finally, in the present study, we were able to successfully complete the life cycle of *O. peruviansus* under laboratory conditions using rabbit to feed ticks. This is useful because the manipulation of the natural host (*D. rotundus*) is complex and dangerous for the researcher (bite risk, pathogen transmission). The selected environmental conditions, host and photoperiod are important factors that must now be considered to better understand the factors that affect the developmental times of this tick.

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