


Life cycle of *Amblyomma mixtum* (Acari: Ixodidae) parasitizing different hosts under laboratory conditions

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Abstract *Amblyomma mixtum* is a tick species in the *Amblyomma cajennense* complex. The known geographic range of *A. mixtum* extends from Texas in the USA to western Ecuador and some islands in the Caribbean. *Amblyomma mixtum* is a vector of disease agents of veterinary and public health importance. The objective of this study was to describe the life cycle of *A. mixtum* under laboratory conditions. Bovines, rabbits and sheep were infested with larvae, nymphs, and adult ticks under controlled conditions to assess several biological parameters. Eggs, larvae, nymphs and adults were kept in an incubator (27 °C temperature

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and 80% relative humidity) when they were off the host. The average life cycle of *A. mixtum* was 88 and 79 days when fed on rabbits and cattle, respectively. Sheep were found to be unsuitable because no ticks attached. The rabbit is a more practical host to maintain a colony of *A. mixtum* under laboratory conditions. The data from this study can be considered as an example for the life cycle of *A. mixtum*. However, caution must be exercised when making comparisons to the biology of *A. mixtum* in its natural habitat.

Keywords Bovine · Rabbit · Laboratory colony · Host suitability · Life cycle

Introduction

Ticks and tick-borne diseases affect humans and animals. Of the approximately 900 extant tick species described worldwide, those in the Ixodidae are the most diverse, comprising approximately 692 species (Nava et al. 2009; Sonenshine and Roe 2014).

Several species in the genus *Amblyomma*, one of 12 genera in the Ixodidae, are of veterinary and public health importance (Labruna et al. 2009; Guzman-Cornejo et al. 2011), including *Amblyomma mixtum* (Nava et al. 2014), which has a three-host life cycle (parasitizing humans, livestock, and wildlife) and is a vector of disease agents (Parola et al. 2005; Castro et al. 2015).

Following the original description by Koch (1844) based on specimens from Mexico, *A. mixtum* was reinstated as a species in the *A. cajennense* complex that includes *A. cajennense* sensu stricto (s.s.), *A. mixtum*, *Amblyomma tonelliae*, *Amblyomma interandinum*, *Amblyomma patinoi* and *Amblyomma sculptum* (Nava et al. 2014). The geographic range of *A. mixtum* extends from Texas in the USA to western Ecuador and some islands in the Caribbean, including Cuba and Trinidad and Tobago (Estrada-Pena et al. 2014; Nava et al. 2014; Beck and Orozco 2015). In some parts of Mexico, *A. mixtum* is an economically important ectoparasite of cattle, second only to *Rhipicephalus microplus* (Guzman-Cornejo et al. 2011; Rodriguez-Vivas et al. 2014). Controlling *A. mixtum* infestations in livestock can be challenging because of its euryxenous parasitic habit (Rodriguez-Vivas et al. 2016).

The biology of *A. mixtum* is not yet fully understood. The availability of laboratory tick colonies facilitates the study of tick biology, vector competence, and control technologies (Bouchard and Wikel 2005). However, some of the challenges to establishing and maintaining tick colonies in the laboratory setting include a shortage of adequate facilities, difficulties in maintaining experimental conditions, and high costs (Allan 2014; Thangamani and Bente 2014).

The results of our study on the colonization and life cycle of *A. mixtum* under laboratory conditions are presented here. Because of the complex interactions at the tick-host interface and their influence on tick rearing (Bouchard and Wikel 2005; Gerardi et al. 2013; Allan 2014), three animal species were tested for host suitability under laboratory conditions. The findings are discussed in the context of available data for other laboratory colonies of species in the *A. cajennense* complex.

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Materials and methods

Animal care and use

All experimental procedures involving animals were conducted in accordance with the rules of the Brazilian National Council of Animal Experimentation (CONCEA). Animal research was approved by the Ethics Committee on Animal Use/CEUA at the Federal University of Mato Grosso do Sul, Campo Grande, MS, Brazil (protocol number 699/2015). In this study sheep, bovines and rabbits were used.

Tick rearing facility

The study was performed on the Embrapa Gado de Corte Experimental Farm (20°25′03″S, 54°42′20″W), Animal Health sector, municipality of Campo Grande, state of Mato Grosso do Sul, Brazil, between November 2014 and March 2016.

Tick source and rearing

Amblyomma mixtum engorged females were collected from naturally infested bovines at a farm in Medellin de Bravo, Veracruz, Mexico (19°01′01.6″N, 96°08′14.4″W). Engorged female ticks were weighed using an analytical scale and housed in vials with perforated covers and maintained in B.O.D. incubators at 27 °C and 80% relative humidity and a photoperiod regimen 12:12 (light:darkness). In addition, the other tick life stages (eggs, larvae, and nymphs) were maintained under the same laboratory conditions to collect off-host data. The species was morphologically identified in accordance with the taxonomic characteristics described by Nava et al. (2014).

Hosts

Bovines (*Bos taurus*), rabbits (*Oryctolagus cuniculus*), and sheep (*Ovis aries*) were tested for their suitability as hosts for *A. mixtum* under laboratory conditions because those vertebrate species were reported to be susceptible to natural infestation or suitable laboratory hosts for North American populations formerly referred to as *A. cajennense* (Cooley and Kohls 1944; Drummond and Whetstone 1975). Ten rabbits of the New Zealand breed, with no previous tick contact and without distinction of gender were used. The chambers were fixed with atoxic glue, according to Szabó et al. (1995). The animals were kept in individual cages with free access to food and water.

Three bovines (*Bos taurus*), ranging in age from 8 to 12 months were kept in individual stalls with free access to food and water. The animals, with previous tick contact, were not treated with acaricides for at least 30 days before infestation. Plastic chambers were used as described above in an area on the back of the animal where hair had been clipped to allow tick feeding. Care and use protocols for bovines were adapted for two adult Santa Ines sheep. These animals had previous contact with ticks. A plastic chamber was also glued to a shaved portion of the sheep's back to confine ticks and allow blood feeding. Santa Ines sheep were chosen because they are sensitive to infestation by ticks (Garcia et al. 2014).

Experimental infestations

Approximately 1000 larvae, 90 nymphs and 12 adult ticks (six males and six females), were used per infestation and per animal. Ticks were placed in feeding chambers on the rabbits, cattle and sheep. All ticks used in experimental infestations were between 15 and 20 days in age. Tick fixation takes only a few minutes. Feed chambers were individually inspected daily to collect engorged ticks. All naturally detached engorged ticks from different hosts were removed from the chambers and taken to the laboratory where they were counted, weighed and identified in vials for incubation and maintained in B.O.D. incubators at 27 °C and 80% relative humidity, and a photoperiod regimen 12:12 (light:darkness).

Parameters analyzed

Several biological parameters were assessed in larvae, nymphs, and engorged female ticks. The formulae described below were adapted and information from other studies was used to make relevant comparisons to *A. cajennense* sensu lato (s.l.) (Hooker et al. 1912; Drummond and Whetstone 1975; Szabó et al. 1995; Sanavria and Prata 1996; Gerardi et al. 2013). To calculate the biological parameters we performed a weighted average. The duration of the life cycle described by us is related only to feeding, molting, pre-oviposition and incubation, being discarded the period when unfed ticks were allowed to feed.

Larval parameters

The engorgement period was defined as the number of days from larvae infestation on the host until the collection day of detached larvae. To calculate the average duration of the engorgement period we performed a weighted average. The individual weight of engorged larvae was estimated from the total weight of larvae collected divided by the number of larvae collected. The duration of the molting process period was defined as the number of days from larvae detachment from the host until the premolt periods. The molting success was defined as the number of larvae that underwent metamorphosis divided by the number of engorged larvae that were collected multiplied by 100%.

Nymphal parameters

The feeding success was defined as the number of nymphs collected divided by the number of released nymphs multiplied by 100%.

The engorgement period was defined as the number of days from nymph infestation on the host until the collection day of released nymphs. To calculate the average duration of the engorgement period we performed a weighted average. The individual weight of engorged nymphs was defined as the total weight of nymphs collected divided by the number of nymphs collected. The duration of the molting process period was defined as the number of days from nymphs detachment from the host until the premolt periods. The molting success was defined as the number of engorged nymphs that underwent metamorphosis divided by the number of engorged nymphs collected, multiplied by 100%.

Engorged female parameters

The feeding success was defined as the number of engorged females that were collected divided by the number of released females multiplied by 100%. The engorgement period was defined as the number of days from the release of adult ticks on the host until the detachment of engorged females. To calculate the average duration of the engorgement period we performed a weighted average. Engorged female weight was determined by weighing each adult female. The pre-oviposition period was defined as the number of days from detachment until the beginning of oviposition. Egg mass weight was defined as the total egg mass deposited by the engorged female. The egg incubation period was defined as the number of days from the beginning of oviposition until larvae eclosion. Larvae eclosion percentage was determined by visual estimation and calculated from the average obtained by three examiners (Szabó et al. 1995). The efficiency index of the conversion of food reserve into eggs was defined as egg mass weight divided by engorged female weight multiplied by 100%.

Descriptive statistics were applied to summarize and describe the biological data. The life cycle parameters of *A. mixtum* obtained in the laboratory were analyzed using BioEstat 5.0 software, with Kruskal–Wallis and Mann–Whitney tests ($\alpha = 0.05$).

Results

Bovines and rabbits were more suitable than were sheep as hosts for *A. mixtum* under laboratory conditions. Sheep were found to be unsuitable because no ticks attached.

Infestation on rabbits

It took 88 days on average for *A. mixtum* to complete its life cycle when feeding on rabbits under laboratory conditions. Thus, approximately 4.1 generations/year could be produced under the laboratory conditions used in this study. Biological parameters obtained from larvae and nymphs were listed in Tables 1 and 2, respectively. The values of reproductive parameters for females are listed in Table 3.

Table 1 Mean (\pm SD; range in parentheses) biological parameters of *Amblyomma mixtum* larvae feeding on rabbit or bovine hosts under laboratory conditions

Host	Weight (mg)	Period (days)		Percentage Molting
		Feeding	Molting	
Rabbit	0.73 \pm 0.6 ^a (0.65–0.78)	6.3 \pm 2.4 ^a (3–12)	12.6 \pm 2.3 ^a (9–17)	74.6 \pm 16.3 ^a (60–96.8)
Bovine	0.96 \pm 0.3 ^b (0.77–1.41)	5.5 \pm 1.2 ^b (4–7)	11.5 \pm 1.2 ^b (10–13)	90.5 \pm 6.9 ^b (80.4–96)

Means within a column followed by different letters are significantly different ($p < 0.05$)

Table 2 Mean (\pm SD; range in parentheses) biological parameters of *Amblyomma mixtum* nymphs feeding on rabbit and bovine hosts under laboratory conditions

Host	Yield (%)	Weight (mg)	Period (days)		Percentage
			Feeding	Molting	Molting
Rabbit	65 \pm 28.2 (27.8–92.9)	12.8 \pm 2.7 (9.1–15.2)	7.45 \pm 2.9 (3–13)	13.5 \pm 2.3 (9–17)	92.7 \pm 14.5 (71–100)
Bovine	43.3 \pm 49.3 (10.3–100)	15.6 \pm 1.7 (13.7–17)	6 \pm 2.5 (4–10)	12.6 \pm 2.4 (10–16)	92 \pm 10.5 (80–100)

Infestation on cattle

The average life time of *A. mixtum* using bovine hosts under laboratory conditions was 79 days. Thus, approximately 4.6 generations/year could be produced under the laboratory conditions used in this study. For larvae and nymphs, the mean values obtained were listed in Table 1 and Table 2, respectively. The values of reproductive parameters for females are listed in Table 3.

Discussion

This is the first study to report data on the life cycle of *A. mixtum* under laboratory conditions on three different hosts. This tick species was originally described by Koch (1844) and was reinstated by Nava et al. (2014). Ticks in the genus *Amblyomma* are represented by 26 species in Mexico, and *A. mixtum* is one of the most widely distributed and prevalent species parasitizing livestock, wildlife and humans (Guzman-Cornejo et al. 2011; Rodriguez-Vivas et al. 2016).

The study of *A. mixtum* as a member of the *A. cajennense* complex is important because previous information related to *A. mixtum* in North America was reported under the *A. cajennense* specific name, including data for colonies maintained in several North American laboratories that did not highlight the potential variations between the six species that are part of the complex (Hooker et al. 1912; Cooley and Kohls 1944; Drummond and Whetstone 1975).

Cattle and sheep can be naturally infested with *A. cajennense* s.l. (Guglielmono and Nava 2006; Alvarez and Bonilla 2007; Gonzalez-Ceron et al. 2009; Ramos et al. 2016). Rabbits are small laboratory animals that are commonly used to maintain colonies of ticks (Sanavria and Prata 1996). The laboratory results presented here confirm field observations suggesting that *A. mixtum* is a generalist host. Based on our findings, rabbits may be the preferred species for long-term maintenance of *A. mixtum* colonies because of their small size, which makes them relatively easier to handle and more convenient with regard to the laboratory facilities required for housing and husbandry costs. And also because all instars of this tick specie had satisfactory results when submitted to feeding on rabbits.

According to the results of this study, the life cycle of *A. mixtum* under laboratory conditions was 88 days when infested on rabbits and 79 days when infested on cattle, unlike that presented by Almazán et al. (2016), this authors reported a life cycle for this species of tick from 133 to 193 days. For pre-oviposition, the present study reports a

Table 3 Mean (\pm SD; range in parentheses) reproductive parameters of *Amblyomma mixtum* females feeding to repletion on rabbit or bovine hosts under laboratory conditions

Host	Yield (%)	Weight (mg)		Period (days)			Percentage	
		Female	Eggs	Feeding	PRE-oviposition	Incubation	Larvae hatching rate	Reproductive efficiency
Rabbit	81.1 \pm 20.0 (60–100)	550.6 \pm 78.6 (462–612.3)	302.5 \pm 38.1 (259.4–332.2)	10 \pm 2.4 (6–15)	5.5 \pm 1.2 (4–8)	33.1 \pm 3.7 (27–39)	68.2 \pm 30.3 (51.7–82)	55 \pm 0.94 (54.3–56.1)
Bovine	40 \pm 0 (20)*	237 \pm 10.6 (229.5–244.5)	146.7 \pm 3.1 (144.5–148.9)	6 \pm 0 (6)*	8 \pm 0 (8)*	30 \pm 0 (30)*	47.5 \pm 3.5 (45–50)	61.9 \pm 1.4 (60.9–63)

* The data presented in these variables show that the ticks were collected on the same day

significantly shorter period than those obtained by Almazán et al. (2016), which showed a variation of 25–29 days for the same parameter, with the cattle as hosts.

Under the same conditions of the present study Almazán et al. (2016) reported an incubation period similar to the results presented by us, as well as a similarity in the engorgement time of all instars of this species of tick in both studies. However different values were reported for the duration of the molting process of nymphs and adults, in which these authors obtained a greater period for this parameter.

In relation to the weight of engorged ticks recovered as well as the respective masses of eggs obtained, the values presented here are significantly lower than those obtained by Almazán et al. (2016). However, the food conversion rate of *A. mixtum* in the present study has a difference of 8.6% more compared to Almazán et al. (2016).

The hypotheses for the differences between the parameters analyzed between larvae, nymphs and adults in the present work in comparison to the results obtained by Almazán et al. (2016) can be several factors, such as, management condition, different breeds of cattle used as hosts, climatic conditions of the study sites and, season of the year.

Our results were similar to those reported for *A. mixtum* (reported as *A. cajennense*) in Texas (Hooker et al. 1912; Strickland et al. 1976). Nymphs of what is now *A. mixtum* from Texas took 3–13 days to engorgement (Strickland et al. 1976), whereas, in our study, nymphs completed feeding after 4–10 days on cattle, and 3–13 days on rabbits.

The acquisition of data for the life cycle of *A. mixtum* under laboratory conditions provided an opportunity for comparative analyses with other tick species of the *Amblyomma cajennense* complex and other *Amblyomma* species under laboratory conditions.

Considering the data reported by Tarragona et al. (2015), *A. tonelliae* under laboratory conditions, when evaluated in different photoperiods before and after feeding, presented a pre-oviposition period of 7–17 days when rabbits were used as hosts. For the *A. mixtum* infesting the same host species, in the present study, the pre-oviposition period varied from 4 to 8 days.

Amblyomma tonelliae engorged female weight varied from 291 to 791 mg, while the *A. mixtum* engorged female weight when infesting rabbits varied from 462 to 612.3 mg, while when infesting bovines varied from 229.5 to 244.5 mg. The incubation period for *A. tonelliae* varied from 36 to 45 days, and for *A. mixtum* varied from 27 to 39 days when infesting rabbits.

Lisboa et al. (1998) reported that *A. sculptum* larvae feeding on rabbits required three to seven days to engorgement. Prata et al. (1996), in a study with *A. sculptum* nymphs feeding on rabbits, reported a recovery rate of 53.4% for nymphs, an engorgement period of 5 days, and a 95% molting success. Similar values to the results found in this study with *A. mixtum*.

Regarding the adult ticks, yield values and reproductive efficiency for *A. mixtum* were similar to the values reported for other tick species from genre *Amblyomma* under laboratory conditions (Troughton and Levin 2007; Olegario et al. 2011; Faccini et al. 2010; Labruna et al. 2004).

Comparisons of biological parameters across the life stages of *Amblyomma* reared in the laboratory show the complex adaptations associated with a three-host life cycle, which may be affected by biotic and abiotic factors (Szabó et al. 2009).

The colony of *A. mixtum* provides an opportunity to produce a continuous supply of ticks for further research on their biology, role as a vector of disease agents, and control technologies. This is especially important in parts of North America where *A. mixtum* is a vector of veterinary and public health importance, including Mexico, where laboratory colonies are not available (Gonzalez-Ceron et al. 2009; Illoldi-Rangel et al. 2012; Alonso-Diaz et al. 2013).

Significant differences have been reported in biological parameters between conspecific ticks under field conditions and those reared in the laboratory (Yu et al. 2010; Meng et al. 2014). The results showed that rabbits and bovines can be a host to maintain this tick species in laboratory, the data from this study conducted under laboratory conditions can be considered as an example for the life cycle of *A. mixtum*. However, caution must be exercised when making comparisons to the biology of *A. mixtum* in its natural habitat.

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Compliance with ethical standards

Conflict of interests The authors declare to have no conflict of interest.

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