Lack of Parthenogenesis by *Amblyomma cajennense* (Acari: Ixodidae)

Carolina MV de Freitas, Romário C Leite, Cristina ML Lopes, Daniel S Rodrigues, Gustavo Fontes Paz, Paulo R de Oliveira+

Laboratório de Ectoparasitoses, Escola de Veterinária, Universidade Federal de Minas Gerais, Av. Antônio Carlos 6627, 30161-970 Belo Horizonte, MG, Brasil

Some reproductive parameters of adult stages of Amblyomma cajennense *ticks were studied. The capacity of virgin females to reproduce by parthenogenesis was evaluated, during an experimental infestation, in absence of males, on a horse* (Equus cabalus)*. Ticks were spread either completely free or in limited sites on the body of the animal. The engorged virgin females showed longer feeding periods and lighter body weights than those that had been fertilized. Some of these unmated females produced smaller egg masses, which had no embryonary development. On the other hand, females that had been inseminated produced larger egg masses, with normal embryonary development that led to viable larvae. Under the studied conditions,* A. cajennense *females did not reproduce by parthenogenesis.*

Key words: *Amblyomma cajennense* - ticks - Ixodidae - reproduction - parthenogenesis

Knowledge of the reproductive aspects of ixodide ticks is essential for the proposal of efficient programs to control them. The vast majority of tick species is anatogenic and bisexual, i.e., there is a need for feeding and copulation for production of a viable progeny (Oliver 1974).

The life cycles of many parasites are synchronized with environmental conditions favorable for reproduction and growth, or with periods of peak host abundance (Chilton et al. 1992). However, under adverse conditions, individuals may develop adaptive changes in order to overcome challenging situations.

Parthenogenesis is a reproductive phenomenon that has been observed in animals, usually as a result of a forced adaptive process. It is characterized by the embryonic development of female eggs without previous fertilization by the male gamete (Soumalen 1950).

The first study on parthenogenesis in ticks was carried out by Aragão (1912). The author described the biological cycle of *Amblyomma rotundatum*, an ixodide tick found in Brazil that infests cold blood animals and reproduces exclusively by parthenogenesis.

Although the usual reproductive process of ticks is bisexual, some species are able to undergo parthenogenesis, when exposed to challenging situations (Oliver 1971). Parthenogenesis has been described, under these circumstances, for *Ornithodorus moubata* (Davis 1951), *Boophilus microplus* (Stone 1963, Ribeiro & Gonzales 1980, Thompson et al. 1980), *Dermacentor variabilis* (Gladney & Dawkins 1971, Homsher et al. 1984), *Haemaphisalis mageshimaensis* (Saito & Hoogstraal 1973), *A. triguttatum triguttatum* (Guglielmone & Moorhouse 1983), *A. dissimile* (Oliver & Pound 1985), *A. cajennense* (Gunn & Hilburn 1991) and *H. leporispalustris* (Labruna & Leite 1997). In these species, after long feeding periods waiting for males, the females produce small egg masses with low hatching rates, which result in weak and unlivable larvae.

Under normal conditions, *A. cajennense* reproduces bisexually and the copulation occurs exclusively on the host. Although its biological and ecological aspects have been well studied, little is known about its reproductive behavior. Gunn and Hilburn (1991) in Texas (EUA) reported the ability of *A. cajennense* females, inoculated in Hereford cattle, to reproduce by parthenogenesis. Those females were originated from the third generation of a standart laboratory colony and were karyotypically characterized as normal diploid.

Considering the economic importance of *A. cajennense* and its implications in public health, it is important to investigate in details its reproductive aspects aiming to propose efficient preventive measures. Therefore, the present study had the objective to evaluate the ability of *A. cajennense* virgin females to reproduce by parthenogenesis, during experimental infestations on horses in absence of *A. cajennense* males.

MATERIALS AND METHODS

The experiment was carried out at the Veterinary Hospital and in the Laboratory of Endo and Eco Parasitic Diseases of the Veterinary School (Universidade Federal de Minas Gerais, Belo Horizonte, Brasil).

In order to obtain adult stages of *A. cajennense*, engorged nymphs were collected from one horse and were individually placed in flasks, which were incubated at 27 ± 2°C and 75% relative humidity (BOD), until completion of ecdisis.

In this experiment a male horse (*Equus caballus*), Mangalarga Marchador breed, of approximately 4 years

This study was sponsored by Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil.
+Corresponding author and study supervisor. Fax: +55-31-3499.2080. E-mail: proliver@vet.ufmg.br
Received 14 November 2001
Accepted 4 July 2002

of age was kept isolated in a stall, receiving food and water roman.

In order to isolate and limit more efficiently the sites of tick infestations, feeding chambers (35 x 20 cm) were prepared using cotton fabric, in a rectangular frame, that allowed the access for tick manipulation. This methodology was an adaptation of the technique previously described by Sanavria et al. (1996).

Previously to infestation, the skin surrounding the feeding chamber was depilated. Following the methodology described by Neitz et al. (1971) and Sanavria et al. (1996), special glue ("pasta ulna") was used to adhere the chamber to the skin.

Each feeding chamber, placed on the left flank of the animal, received 40 virgin unfed 20-days-old females of *A. cajennense*, which constituted the test group (TG). Another feeding chamber, placed on the right flank, received 32 females and 15 males of *A. cajennense*. These were also 20-days-old, virgin, and unfed individuals, and constituted the control group (CG). A single infestation was carried out in each experimental group.

Through out the parasitic stage, the number of feeding females was recorded daily. The detached females, showing either complete or partial engorgement, were taken to the laboratory, where they were individually weighed, placed in Petri dishes, and incubated under BOD conditions, as previously described. The females were daily inspected and, at the end of the oviposition period, the masses produced were weighed and placed into sterile plastic syringes and incubated under BOD conditions.

For both groups (TG and CG) the following parameters were recorded: fixation and recovering rates, engorgement period, weight of engorged females, preoviposition, and oviposition periods, egg mass, egg incubation period, and hatching rates. The reproductive efficiency index and the efficiency in converting eggs were calculated according to the methodology described by Drummond and Whetstone (1975). The total number of eggs produced by each female was estimated according to Labruna et al. (1997). Data were analyzed by the t student test and correlation.

RESULTS

The percentage of fixation of females observed in the TG was 77.5% and the recovering rate was 20%. These values were significantly lower ($p < 0.05$) than those obtained for females in the CG (96% and 78.1%, respectively).

Females in the TG had an engorgement period of 30.63 ± 10.78 days, which was significantly higher than that observed in the CG (11.24 ± 1.86 days). Although the unmated females in the TG had long feeding periods, the ones that were able to engorge weighed 223 ± 193 mg. This value was significantly lower ($p < 0.01$) than that recorded for fertilized females in the CG (887 ± 71 mg).

There was a negative correlation between the parasitic period and the weight of unmated females in the TG ($n = 4$; $r = -0.9502$; $p < 0.05$). Such correlation was not observed in the CG.

The Table summarizes the results obtained from females in both groups (TG and CG). In the TG group only the females that had engorged and produced eggs were considered.

The mean preoviposition period observed for virgin females in the TG was 2.5 days and it was significantly lower than that observed for fertilized females in the CG (5.64 days).

Only four (10%), among 40 inoculated females and among eight (20%) recovered females from the TG were able to produce eggs. In contrast, all engorged females in the CG (25) were able to produce eggs.

The virgin and engorged females in the TG that did not lay eggs had a mean weight of 71 mg. This value is significantly lower ($p < 0.05$) than that observed for females in the CG (373 mg), which underwent oviposition.

The mean oviposition period observed for unmated females in the TG was 24.75 days, ranging from 19 to 33 days. This period was significantly shorter ($p < 0.5$) than that recorded for females from the CG (28.52 days).

The eggs produced by females from the TG were dark brown and opaque, but became black and dehydrated as they aged. In contrast, the eggs produced by fertilized females from the CG were bright and light brown colored.

TABLE

Biological parameters of *Amblyomma cajennense* females feeding on a horse in absence (TG) or presence (CG) of *A. cajennense* males

	N = 4 Absence of males (TG)			N = 25 Presence of males (CG)		
Biological parameter	Mean	Range	Standard Deviation	Mean	Range	Standard Deviation
Preoviposition period (days)	2.5	0-4	1.91	5.64	3-8	0.95
Oviposition period (days)	24.75	19-33	6.02	28.52	23-34	2.82
Egg mass (mg)	138	31-272	100	485	293-593	71
Number of eggs per oviposition	2604	585-5132	1404.31	9152	5528-11189	1336.11
Incubation period (days)	NE	NE	NE	37.64	34-44	3.12
Hatch of eggs (%)	NE	NE	NE	90.6	75-95	5.46
REI = number of eggs/engorged weight (g)	7187	1671-11493	4133	10352	6303-11582	1271
CEI = egg mass (g)/engorged weight (g)	0.38	0.09-0.61	0.22	0.549	0.334-0.614	0.07

NE: no eclosion; REI: Reproductive Efficiency Index; CEI: Conversion Efficiency Index

The mean egg mass and the mean number of eggs produced by females in the TG were respectively 138 mg and 2,603.77 eggs. These females had egg masses significantly lighter ($p < 0.01$) and smaller than those produced by females in the CG (485.08 mg and 9.52 eggs respectively).

The unmated females in the TG showed a Reproductive Efficiency Index (REI) of 7,187 and a Conversion Efficiency Index (CEI) of 0.380. These values were significantly lower ($p < 0.01$) than those estimated for inseminated females in the CG (10,352.4 and 0.549, respectively).

Through out the experimental period, no embryonic development was observed in eggs originated from females in the TG. Their eggs became dehydrated and darkened and did not lead to eclosion.

All *A. cajennense* females recovered from the CG produced eggs that presented high levels of embryonic development and had a mean eclodibility rate of 90.6%. As shown in the Table, the mean incubation period for this group was de 37.64 days. The TG did not show any egg eclodibility.

DISCUSSION

In the present study, we report a low recovering rate of virgin females of *A. cajennense* feeding in absence of males. Gladney (1971), Papas and Oliver (1972), Norval et al. (1989), and Labruna (1996) also reported significantly lower recovering rates of females of other tick species, in absence of males during the feeding period.

Absence of males during the feeding period led to a longer parasitic stage of virgin females in the TG (30.63 ± 10.78 days). This result agrees with those reported by Gunn and Hilburn (1991), who observed a mean parasitic stage of 29.37 days for *A. cajennense* females, from a standart laboratory colony, feeding on cattle in absence of males. Stone (1963), Gladney and Dawkins (1971), Saito and Hoogstraal (1973), Ribeiro and Gonzales (1980), Nitiamoa-Baidu (1987), Falk-Vairant et al. (1994) and Labruna (1996), also reported, for other species of ticks, longer engorgement periods of females feeding in absence of males. According to Balashov (1972), if bisexual females have not been fertilized they remain feeding on the host for unlimited periods of time waiting for males. This behavior of unmated females can be related, according to Rosel-Davis and Coons (1989), to the fact that vitelogenesis is directly dependent on long feeding periods.

The body mass was also significantly influenced by absence of fertilization, since females in the TG weighed in average 223 mg. Low body weights of virgin females of other tick species feeding in absence of males have also been described by Stone (1963), Gladney and Dawkins (1973), Brown and Stenner (1982), Guglielmone and Moorhouse (1983), Homsher et al. (1984), and Labruna (1996). Besides, Gunn and Hilburn (1991) also observed a negative correlation between the body weight and duration of the engorgement period of non-fertilized females of *A. cajennense.* According to the authors, the highest body weights were observed in females showing the shortest engorgement periods.

Females in the TG had a preoviposition period significantly lower than that observed for females in the CG. Labruna (1996) and Gladney and Dawkins (1971) reported shorter preoviposition periods in unmated females of *H. leporispalustris* and *D. variabilis* than those observed in inseminated females of the same species. Saito and Hoogstraal (1973) and Ribeiro and Gonzales (1980), studying *H. mageshimaensis* and *B. microplus* ticks did not observed significant differences between the preoviposition periods of fertilized and non-fertilized females.

The low number of females that underwent oviposition in the TG observed in the present study is in agreement with results reported by Ribeiro and Gonzales (1980). These researches observed that the main factor affecting oviposition of *B. microplus* virgin females was the decrease of body mass. In the present study, the mean body weight of females that did not produce eggs in the TG was 71 mg. This value was significantly lower than that observed for females that underwent oviposition (373 mg) in the same group.

Saito and Hoogstraal (1973) did not find significant variations of oviposition periods between fertilized and non-fertilized females of *H. magehimaensis*. However, in the present work, absence of fertilization resulted in significant decrease of the oviposition period of females in the TG. Labruna (1996) also observed that presence or absence of males determined considerable variations of oviposition periods of *H. leporispalustris* females.

The mean oviposition weight, the mean number of eggs produced and, consequently, the REI and the CEI observed for females in the TG were influenced by absence of insemination. Gladney and Dawkins (1973), Saito and Hoogstraal (1973), Londt (1976), and Ntiamoa-Baidu (1987) also reported, for other tick species, that females feeding in absence of males produced significant lower number of eggs than those produced by fertilized females. Labruna (1996) observed that *H. leporispalustris* females that had engorged without insemination showed lower REI than those females in contact with males during the feeding period. These results are in agreement with observation made by Ribeiro and Gonzales (1980), who reported a positive correlation between body weight of *B. microplus* engorged females and number of eggs produced in absence of males ($n = 55$; $r = 0.96$ and $p < 0.05$).

The results of the present study point to the importance of insemination and presence of males in the reproductive performance of heterosexual ixodide females. Among the females feeding in absence of males, very few were able to produce eggs, which had no embryonic development and were, therefore, unable to eclode. Homsher et al. (1984), evaluating the occurrence of parthenogenesis in *D. variabilis* females, reported a mean egg eclodibility of 5.6%. On the other hand, Londt (1976) verified that *B. microplus* virgin females were able to produce eggs, but only 0.8% hatched. Gunn and Hilburn (1991) reported that eggs from non-inseminated *A. cajennense* females had a mean eclodibility rate bellow 1%. However, in all these studies the majority of larvae originated from those eggs were unable to feed and rarely developed into the adult stage.

No accidental parthenogenesis was observed in *A. cajennense* females feeding on a horse, in absence of *A.*

cajennense males. Although these females had long feeding periods, most of them failed to engorge, and those that did so had low body weights. Some of these females laid few eggs, but these had no embryonic development and, consequently, did not lead to eclosion of larvae.

REFERENCES

Aragão BH 1912. Contribuição para sistematica e biolojia dos ixódidas. Partenogênese em carrapatos. *Mem Inst Oswaldo Cruz 4*: 96-119.

Balashov YS 1972. Bloodsucking ticks (Ixodoidea) - Vectors of diseases of man and animals. *Misc Pub Entomol Soc Am 8*: 337.

Brown SJ, Stenner T 1982. Regulation of feeding and ovipositional success of *Amblyomma americanum* tick. *Experientia 38*: 1060-1061.

Chilton NB, Andrews RH, Bull CM 1992. Delayed mating and the reproductive fitness of *Aponomma hydrosauri* (Acari: Ixodidae). *Int J Parasitol 22*: 1197-1200.

Davis GE 1951. Parthenogenesis in the argasid tick *Ornithodoros moubata* (Murray, 1877). *J Parasitol 73*: 99-101.

Drummond RO, Whetstone TM 1975. Ovoposition of the cayenne tick, *Amblyomma cajennense* (F.), in the laboratory. *Ann Ent Soc Am 68*: 214-216.

Falk-Vairant J, Guerin PM, Bruyne M, Rohrer M 1994. Some observations on mating and fertilization in the cattle tick *Boophilus microplus*. *Med Vet Entomol 8*: 101-103.

Gladney WJ 1971. Mate-seeking by female *Amblyomma maculatum* (Acarina: Ixodidae) on a bovine. *Nature 232*: 401-402.

Gladney WJ, Dawkins C 1971. Parthenogenetic reproduction by *Dermacentor variabilis* (Acarina: Ixodidae). *Ann Ent Soc Am 64*: 1285-1289.

Gladney WJ, Dawkins CC 1973. Experimental interspecific mating of *Amblyomma maculatum* and *Amblyomma americanum*. *Ann Ent Soc Am 66*: 1093-1097.

Guglielmone AA, Moorhouse DE 1983. Copulation and successful insemination by unfed *Amblyomma triguttatum triguttatum* Koch. *J Parasitol 69*: 786-787.

Gunn SJ, Hilburn LR 1991. Parthenogenesis and karyotypic evolution in the cayenne tick, *Amblyomma cajennense* (F.): model for the production of karyotypic changes through a parthenogenetic pathway. *J Med Entomol 28*: 340-349.

Homsher PJ, Soneshine DE, Mason SN 1984. Thelytoky in the American dog tick, *Dermacentor variabilis* (Acari: Ixodidae). *J Med Entomol 21*: 307-309.

Labruna, MB 1996. *Aspectos da Biologia de Haemaphysalis leporipalustris* (Pacckard, 1869), MSc Thesis, Escola de Veterinária, UFMG, Belo Hoerizonte, 132 pp.

Labruna MB, Leite RC 1997. Reproductive aspects of *Haemaphysalis leporispalustris*. *Mem Inst Oswaldo Cruz 92*: 373-376.

Labruna MB, Leite RCL, Oliveira, PR 1997. Study of the weight of eggs from six ixodid species from Brazil. *Mem Inst Oswaldo Cruz 92*: 205-207.

Londt JGH 1976. Fertilization capacity of *Boophilus decoloratus* (Koch, 1884) (Acarina: Ixodidae). *Onderstepoort J Vet Res 43*: 143-146.

Neitz WO, Boughton F, Walters HS 1971. Laboratory investigations on the life-cycle of the karoo paralysis tick (*Ixodes rubicundus* Neumann, 1904). *Onderstepoort J Vet Res 38*: 215-224.

Nitiamoa-Baidu Y 1987. *Rhipicephalus simpsoni* (Acari: Ixodidae) development under controlled conditions. *J Med Entomol 24*: 438-443.

Norval RAI, Colborne J, Tannock J, Mackenzie PKL 1989. The life cycle of *Amblyomma tholloni* Neumann, 1989 (Acarina:Ixodiae) under laboratory conditions. *Vet Parasitol 7*: 255-263.

Oliver Jr JH 1971. Parthenogenesis in mites and ticks (Arachnida: Acari). *Am Zoologist 11*: 283-299.

Oliver Jr JH 1974. Symposium on reproduction of arthropods of medical and veterinary importance. Reproduction of ticks (Ixodoidea). *J Med Entomol 11*: 26-34.

Oliver Jr JH, Pound JM 1985. Cytogenetic of ticks (Acari: Ixodoidea). Cromosomes and laboratory biology of *Amblyomma dissimile*. *J Med Entomol 22*: 459-463.

Pappas PJ, Oliver Jr JH 1972. Reproduction in tick: analysis of the stimulus for rapid and complete feeding of female *Dermacentor variabilis*. *J Med Entomol 9*: 47-50.

Ribeiro VLS, Gonzales JC 1980. A partenogênese no *Boophilus microplus* (Canestrini, 1887). *Arq Fac Vet UFRGS 8*: 93-108.

Rosell-Davis R, Coons LB 1989. Relationship between feeding, mating, vitellogenin production and vitellogenesis in the tick *Dermacentor variabilis*. *Exp Appl Acarol 7*: 95-105.

Saito Y, Hoosgstraal H 1973. *Haemaphysalis (Kaseriana) mageshimaensis* sp. N. (Ixodoidea: Ixodidae), a Japanese deer parasite with bisexual and parthenogenetic reproduction. *J Parasitol 59*: 569-578.

Sanavria A, Prata MCA, Morais MC, Alonso LS 1996. Determinação de alguns parâmetros biológicos de *Amblyomma cajennense* (Fabricius, 1787) (Acari: Ixodidae) em infestação artificial de equinos. *Arq Fac Vet UFRGS 24*: 79-86.

Soumalen E 1950. Parthenogenesis in animals. *Adv Genet 3*: 193-253.

Stone BF 1963. Pathenogenesis in the cattle tick, *Boophilus microplus*. *Nature 200*: 1233.

Thompson GD, Davey RB, Osburn RL, Cruz D 1980. Longevity and fertilization capacity of males and parthenogenesis in females of *Boophilus annulatus* and *B. microplus. J Econ Entomol 73*: 378-380.