Comunicación corta

AN AUTOMATIC SYSTEM TO FEED TICKS THROUGH MEMBRANES

R. de la Vega*, A. Camejo* and A.H. Fonseca**

*LABIOFAM, Ave Independencia Km 161/2, Ciudad Habana, Cuba. E-mail: delavega@infomed.sld.cu **Lab Enfermedades Parasitárias, Veterinária, Universidade Federal Rural do Rio de Janeiro, Km 47, Seropédica,RJ, Brasil. Email: adivaldo@ufrrj.br

ABSTRACT: The ability to artificially feed ticks in laboratory has great potential importance for the study of the interrelation tick-hemoparasite, the effect of systemic acaricides and vaccines, as well as for facilitating a deeper understanding of ticks’ biology. Furthermore, such a technique would save money and time, and would also ameliorate the ethical problems that arise from the use of natural hosts in experimental settings. The main objective of this work was to obtain a fully automatic artificial feeding system. Recently moulted females and mature males (10 to 20 couples) of *Boophilus microplus* were put together in feeding chambers. A second species, *Amblyomma cajennense*, was also used. These ticks were fasted for four months while being incubated at 28 °C and 80% relative humidity. Males were put in the device a week before females. The feeding chamber is a piece of metal 90X90X15 mm with a cylindrical hole of 50 mm diameter and 9 mm deep. The membrane was made of thin paper, reinforced with a solution of silicone. Bovine hair, ticks’ exuviae and faeces, and CO2 were used as phagostimulants. Gentamicine and nistatine were added to prevent bacterial and fungal growth. Blood was obtained under sterile conditions and stored in a refrigerator at 7 to 8°C. Blood temperature within the feeding chamber was maintained between 37 and 38°C. A peristaltic pump transferred blood from the refrigerator to the feeding chamber at a rate of 15-20 drop/minute, every 4 to 8 hours. The system worked automatically for more than three weeks. Partially engorged females were obtained, demonstrating the efficacy of this new automatic feeding system.

(Key words: ticks; artificial feeding; membranes)

SISTEMA AUTOMÁTICO DE ALIMENTACIÓN ARTIFICIAL DE GARRAPATAS A TRAVÉS DE MEMBRANAS

RESUMEN: La alimentación artificial de garrapatas en el laboratorio tiene una gran importancia para el estudio de la interrelación garrapatas-hemoparásito, la acción de acaricidas sistémicos y vacunas, así como para profundizar el conocimiento de la biología de estos ácaros. Además, el método serviría para ahorrar dinero, tiempo y atenuar problemas éticos al disminuir la utilización de animales en la experimentación. El principal objetivo del presente trabajo fue obtener un sistema de alimentación artificial completamente automatizado. Hembras de *Boophilus microplus* recientemente mudadas y machos maduros sexualmente, en número de 10 a 20 parejas, se pusieron juntos en cámaras de alimentación artificial. Otra especie empleada fue *Amblyomma cajennense*. Estas garrapatas estuvieron en ayuno por cuatro meses incubadas a 28°C y 80% de humedad relativa; en este caso los machos se colocaron en la cámara de alimentación una semana antes que las hembras. La cámara de alimentación está constituida por una pieza de metal de 90x90x15 mm con un orificio cilíndrico de 50 mm de diámetro y 9 mm de profundidad. La membrana fue confecionada con papel fino reforzada con una solución de silicona. Como fagosestimulantes se emplearon pelo de bovino, exuvias y heces de garrapatas y CO2. Para prevenir el crecimiento de bacterias y hongos se emplearon gentamicina y nistatina. La sangre fue extraída de los bovinos en forma aséptica y guardada en un refrigerador a 7-8 °C. La temperatura de la sangre en la cámara de alimentación estuvo entre 37 y 38 °C. La sangre fue impulsada por una bomba peristáltica a la velocidad de 15-20 gotas/minuto, cada 4 a 8 horas de intervalo, de la sangre del refrigerador a la cámara de alimentación. El sistema fue capaz de trabajar automáticamente por más de tres semanas. Se obtuvieron hembras parcialmente repletas.

(Palabras clave: garrapatas; alimentación artificial; membranes)
The ability to artificially feed ticks in laboratory has great potential importance for the study of the interrelation tick-hemoparasite, the effects of systemic acaricides and vaccines, as well as for facilitating a deeper understanding of ticks' biology. Furthermore, such a technique would save money and time, and would also ameliorate the ethical problems that arise from the use of natural hosts in experimental settings.

Many attempts have been made to feed and raise ticks in laboratory. Micropipettes, hen egg membranes, mouse, cattle, pig and bird skins, Baudruche membrane and artificial membranes (silicone, parafilm, latex, etc) have been tried with different species of ticks (1,2,4,5,6,8,10,11,12,15).

One of the most important barriers to the development of an artificial feeding system is the need to frequently manipulate the device to effect blood changes. There are opinions of some of the most experienced researchers in the field: "...changing the blood manually...is inefficient, tedious and time-consuming" (13). "An additional modification that can be introduced to the system is the attachment of a mechanical device to enable circulation of blood, obviating the need to change blood, physically every 8-10 hours" (14).

The main objective of this work was to obtain a fully automatic artificial feeding system. Such a system has now been developed by applying significant improvements to an early apparatus created and then rejected by Kuhnert (7).

Nymphs of *Boophilus microplus* were picked off 13–14 days after artificial larvae infestation of a bovine and incubated at 28°C and 80 % relative humidity to achieve adult moult. Recently moulted females were weighed and put in the feeding chamber immediately after moult; moulted males were discarded. Sexually mature males were obtained from another bovine that had been previously infested. A second species, *Amblyomma cajennense*, was also used. These ticks were fasted for four months at 28 °C and 80% relative humidity. In this protocol, males were put in the feeding chambers a week before females. For both species, from 10 to 20 couples were employed in every experiment.

The feeding chamber is a piece of metal 90X90X15 mm with a cylindrical hole 50 mm diameter and 9 mm deep. The chamber is similar in shape to that used by Kuhnert et al. (9) but in this case it was warmed by a hot plate, not by circulating warm water. Blood was circulated by a peristaltic pump at different time intervals; delivery of blood was controlled by a programmable timer, rather than continuously as in Kuhnert’s device; thus, the amount of blood employed in two weeks is less than half a liter. Blood was obtained under sterile conditions and was stored in a refrigerator at 7 to 8°C. Blood temperature at the feeding chamber was maintained between 37 and 38°C. Sodium Heparin, 10 IU/mL was used as anticoagulant. Gentamicine and nistatine (9) were added at a dose of 100mi/mL to prevent bacterial and fungal growth. The membrane was made of thin paper, reinforced with a solution of silicone. Bovine hair, ticks’ exuviae and faeces and CO2 were used as phagostimulants.

Every 4 to 8 hours a peristaltic pump, at a rate of 15-20 drop/minute, moved blood from the refrigerator to the feeding chamber. Blood was stirred within the feeding chamber and the refrigerator at different intervals for set periods of time by means of two independent programmable timers and two magnetic stirrers. Every time, blood was pumped to the feeding chamber and residual blood was discarded. Blood pressure within the system was kept at atmospheric values. All parts of the system were sterilized by autoclave, before starting experiments.

The salient achievements of this work are:

- The temperature of the feeding system is kept constant between 37 and 38°C
- There is no blood “bleeding” on the membrane
- Ticks’ guts do not rupture since no hypertension is present
- No air bubbles are present since there is no hypotension either
- There is adequate control of bacteria and fungi
- There is an effective control of stirring and pumping periods
- The great majority of ticks (*Amblyomma cajennense* and *Boophilus microplus*) on the membrane are capable of attaching and feeding
- Many couples in the mating position are seen
- There is an excellent stability of the membrane
- The system is able to function automatically for more than three weeks
- Only one manipulation of blood, its disposal, is needed and this is accomplished out of the system

Some pictures (Fig. 1 to 4) of *Boophilus microplus* and *Amblyomma cajennense* during the 5th to 7th days of feeding are shown below. The sizes and weights of...
B. microplus females were very close to those observed in the host (3). However, final engorgement of the ticks is still lacking. Continuing experiments are being conducted to overcome this deficiency.

FIGURA 1. Copula of Boophilus microplus (Magnification 12X)./ Copula de Boophilus microplus (Aumento 12X).

FIGURA 2. Couple of Amblyomma cajennense (Magnification 13X)./ Pareja de Amblyomma cajennense (Aumento 13X).

FIGURA 3. Semi-engorged Boophilus microplus female (Magnification 14X)./ Hembra de Boophilus microplus semi-ingurgitada (Aumento 14X).

FIGURA 4. Group of B. microplus and A. cajennense (Magnification 8X)./ Grupo de B. microplus y A. cajennense (Aumento 8X).
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