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Advances in the artificial feeding of *Ornithodoros moubata* (Acari: Ixodidae) and the follow up of its life cycle after feeding on fetal bovine serum

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ABSTRACT

Ornithodoros moubata was fed artificially through a parafilm membrane with two types of artificial meals. The first type was composed of bovine and horse red blood cells suspended in phosphate buffered saline or fetal bovine serum (FBS). The second type was composed of FBS alone. Engorgement and post engorgement survival rates of the ticks after feeding on the two types of meals were compared and analysed statistically. FBS was found to be superior to red blood cell meals. Eventually two batches of FBS from different manufacturers were used to feed the ticks through artificial membrane. Engorgement and post engorgement survival rates were again compared and analysed statistically. There was no significant difference between the two lots. Finally *O. moubata* was fed on FBS and successful reproductive cycle followed up to the third generation.

INTRODUCTION

The Argasidae tick *Ornithodoros moubata* is a long lived and highly resistant tick. The tick is difficult to control because it is active at night and parasitize both wild and domestic animals in kraals and burrows and hence difficult to notice their infestation (Jongejan and Uilenberg 1994, Ruheta, 1999). *O. moubata* pose a threat to livestock development and in particular to pig industry in the tropics and subtropical areas. The tick transmit African swine fever virus (Osborne and Mellor, 1985), a causal agent of the fatal African swine fever disease. The tick also transmit *Borrelia dutoni* (Novy and Knapp, 1906) which causes African relapsing fever in human. Under natural condition the tick feed on blood, in vitro feeding of the tick and maintenance in the laboratory using blood and other artificial meals had been going on in many parts of the world, thus avoiding the cost of keeping laboratory animals (Hokama et al. 1987, Osborne and Mellor, 1985). The advantages of artificial feeding of ticks include evaluation of diseases transmission mechanism, studies on tick biology, immunology, vaccinology and the control of spread of infection during experiment (Hood et al. 1976, Abbassy et al. 1994). In this study *O. moubata* was fed with different meals through an artificial feeding membrane with the objectives of: (1) Assessing the possibility of using parafilm membrane without phagostimulants in the artificial feeding of *O. moubata*.

(2) Comparison of fetal bovine serum (FBS) and red blood cell (RBC) meals with regard to engorgement and post feeding survival.

(3)Comparison of two batches of FBS.

(4) Evaluation of the tick life cycle after artificial feeding with FBS .

Materials and methods

Ticks used in the artificial membrane feeding

O. moubata (Murray) sensu Walton (1962) ticks used in this study, were from a colony kept at the National Research Centre for Protozoan Diseases (NRCPD) since 1997 (Inoue et al. 2001).

Artificial meals used

Horse red blood cell (HoRBC), bovine red blood cell (BoRBC) and FBS lot number 7M1069 (Bio Whittaker, Maryland, USA) and 244008-28 (Boehringer Mannheim K. K., Australia) were used. In the comparison of RBC and FBS meals, equal volumes of HoRBC and BoRBC i.e 750 µl (50%) were suspended in 750µl of 1M phosphate buffered saline (PBS) at the pH of 7.4 or FBS lot no.7M1069. The two types of RBC meals suspended in PBS/FBS were fed on alternative days and the resulting data were pooled and analysed as a single meal.

Preparation of artificial membrane *apparatus*

The artificial membrane *apparatus* described by Howarth and Hokama(1978) was used with a slight modification. In this study the small (2.5 ml) plastic wells (Becton Dickinson, Meylan Cedex, France) had their bottom removed and fitted with (Parafilm ″M″ ® American Can ™, Chicago, IL. 60631) membrane. Ticks were then placed in the containers so that they can feed through the membrane. The above containers were lowered into a bigger (8 ml) plastic wells (Becton Dickinson, Meylan Cedex, France) in which 1500 µl of meal had been added. The assembled feeding kit with the lower surface of the parafilm membrane in contact with the meal was placed in a water bath-cum-shaker (Eyela, Tokyo Rikakikai Co., Ltd NTS-1300) preheated to 37°C. When RBC meals were used the water bath was shook so as to avoid sedmentation of red blood cells.

Artificial feeding procedure

After preparation of the artificial membrane apparatus, 294 adult ticks were allowed to feed for up to two hours. Engorged ticks were removed and kept in a petri dish lined with filter paper (Whatman International Ltd, Maidstone, England). Ticks that did not feed within two hours were removed and kept separately, such ticks were used in future feeding experiments.

Comparison of artificial meals used and statistical analysis

Engorgement, mortality and post engorgement survival of ticks fed on RBC, FBS (7M1069) and FBS (244008-28) were compared and analysed by chi-square test (Fowler and Cohen, 1992).

Life cycle of *O. moubata* after feeding on FBS 7M106

Engorged ticks were kept inside a petri dish lined with filter paper where they mated in 5 – 10 minutes after feeding, eventually they were maintained collectively in petri dishes at 28° C in darkness in an incubator (SANYO, Japan) at an approximately 50% relative humidity. Egg laying lasted for about two and half weeks, starting from 10 – 13 to 27 – 30 days post feeding. The eggs hatched into larva, the larvae could molt into nymphs without feeding. The nymph stages 1-5 were fed on rabbit using an ear bag technique with

each nymph stage molting to the next stage 7-9 days after feeding. The resulting adults were fed on FBS, mating occurred inside petri dishes after feeding and eggs were laid again at the condition described above. The eggs hatched into larvae and the subsequent nymph stages of the second generation were again fed on rabbit before molting into adults. Similar procedure was repeated for the third generation.

RESULT

Feeding through parafilm ″M″ membrane

It was observed that 11 ticks (69%) out of 16 ticks that were fed on horse and bovine RBC meals and 102 ticks (65%) out of 157 ticks fed on FBS 7M1069 could feed successfully through the membrane (Table 1). Comparison of engorgement between RBC and FBS meals showed no significant difference (x^2 test, $p > 0.05$). The time of feeding to full engorgement varied between 20 minutes to 2 hours with the majority of ticks engorging between 20 minutes and 40 minutes.

Comparison of post feeding survival and mortalities after feeding on RBC and FBS 7M1069 meals

Beside good engorgement rate i.e. (69%), ticks that were fed on RBC meals could not survive well after engorgement because 6 ticks (54.5%) out of 11 engorged ticks died within 1 to three days post feeding. Ticks that fed on FBS 7M1069 showed both good engorgement (65%) and post engorgement survival records. 88 ticks (86.5%) out of 102 ticks that became engorged survived (Table 2). There was a highly significant difference in survival post feeding between the two groups. Ticks that fed on FBS survived better than those fed on RBC meals (x2 test, $p < 0.001$).

Comparison of two batches of FBS

There was no significant difference between engorgement and post engorgement survival between ticks fed on FBS 7M1069 and 244008-28 in the artificial feeding experiments (x2 test, $p > 0.05$) (Table 3 and 4)

Feeding on the Rabbit

Nymph stage 1–5 that were fed on the rabbit became engorged after thirty minutes, few nymphs died inside the ear bag soon after feeding and in the petri dishes 2 – 3 days later.

Life cycle of *O. moubata* after feeding on FBS 7M1069 meal.

After finding that there was no significant difference between the two lots of FBS, eventually FBS 7M1069 was used alone in the artificial feeding experiments that followed. Some ticks died in 24 – 36 hours post feeding, such ticks were easily detected because they changed into blackish –red haemorrhagic colouration, ticks that survived laid viable eggs, the eggs hatched into larvae and the larvae hatched into nymph without feeding. Similar results were reported by Hoogstraal (1956) after finding that the larvae stage did not require a meal to complete a molting cycle.

Table 1. Comparison of engorgement rate after feeding with RBC and FBS meals ticks

Meal	No. of fed ticks	No. of engorged ticks	No. of unengorged
FBS	157	102 (65%)	55 (35%)
RBC	16	11 (69%)	5 (31%)

[a]50% of bovine/horse RBC in 1500µl PBS buffer/FBS.

[b]Tick engorgement was observed within 20 to 40 minutes after application on the artificial membrane apparatus.

Table 2. Comparison of post feeding survival and mortality rate after feeding with RBC and FBS meals

Meal	No. of engorged ticks	No. of survived ticks	No. of died ticks
FBS	102	88 (86.3%)	14 (13.7%)
RBC	11	5 (45.5%)	6 (54.5%)

[a] $p < 0.001$

[b]Survival of ticks were determined within 1 to 3 days after engorgement.

[c]Died ticks were identified from the outer blackish-red hemorrhagic appearance and immobility.

Table 3. Comparison of engorgement rate after feeding with FBS lot No. 7M1069 and 244008-28

FBS lot No.	No. of fed ticks	No. of engorged ticks	No. of unengorged ticks
7M1069	60	39 (65%)	21 (35%)
244008-28	51	35 (69%)	16 (31%)

[a]$p > 0.05$

Table 4. Comparison of survival rate after feeding with FBS lot No 7M1069 and 244008-28

FBS lot No.	No. of engorged ticks	No. of survived ticks	No. of died ticks
7M1069	39	33(85%)	6 (15%)
244008-28	35	31 (89%)	4 (11%)

[a]$p > 0.05$

DISCUSSION

The tick *O. moubata* was successfully fed through a simple parafilm ″M″ membrane without employment of tactile stimuli, majority of the ticks engorged in 20 – 40 minutes, these findings tallies with Hood et al.(1976) who reported time needed for *O. moubata* feeding through exposed membranes of embryonated hen eggs to vary between 10 – 90 minutes. However the results are contrary to previous study by Hokama et al.(1987) who reported that *O. coriaceus* ticks could not feed through parafilm membrane if it was not overlaid by hair or nylon netting which acted as a phagostimulant. Voigt et al.(1993) reported carbon dioxide in a 5 – 10% range and blood temperature above 37°C to enhance attachment and feeding of ticks on artificial feeders. Initially BoRBC and HoRBC suspended in PBS or FBS were used in the artificial membrane feeding. However survival rate of the ticks after feeding was very poor possibly due to the fact that the RBCs which had been stored for some time might have developed toxins due to degradation. Osborne and Mellor (1986) reported that toxic chemical factors in stored blood become progressively more noticeable as the blood age despite storage at - 20°C. They also attributed mortalities after artificial feeding to contamination by microbial agents and tick internal damage as a result of overfeeding. With regard to post feeding survival, FBS was found to be superior to RBCs meals probably due to the fact that FBS is uniform and does not sedment hence avoiding the constant shaking during feeding which is a prerequisite when RBC meals are used. Abbasy et al.(1994) reported that FBS does not coagulate and is free from contaminants. Hood et al.(1976) attributed post blood feeding mortalities of *O. moubata* to many unspecified reasons.

As a result of good survival rate of ticks fed on FBS, the use of RBC meals were terminated and further experiments continued with the use of FBS. Due to the fact that the NRCPD had two batches of FBS we went ahead to find if there was any difference between them. The study showed that there was no

significant difference between FBS lot number 7M1069 and 244008-28 (x^2 test, $p > 0.05$) hence any of the two can be used in the artificial feeding of *O. moubata*, as a result further artificial feeding experiments were continued with FBS 7M1069 alone. Successful reproduction cycles after feeding on the FBS was followed up to the third generation. The artificial feeding experiments could not go beyond the third generation because all ticks were used for another experiment. Mortalities of nymph stages after feeding on the rabbit may be attributed to the fact that *O. moubata* and in particular the nymph stages are delicate hence they can be easily damaged against the ear bag while feeding/engorged if the rabbit shakes the ear vigorously. If the damage is visible externally immediate death usually follows. On the other side if damage has occurred internally e.g. through rupturing of internal organs and internal bleeding, death usually occur 2-3 days post feeding. In order to minimise such mortalities engorged nymphs were removed from the ear bag after every 20-30 minutes until all nymphs were all engorged and collected into a petri dish.

Artificial membrane feeding through a parafilm membrane with FBS 7M1069 or 244008-28 could provide another alternative way of maintaining *O. moubata* in the laboratory with the subsequent advantages of carrying out various studies on tick control options, disease transmission and tick biology with minimum cost.

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