

Raising of Horn Fly *Haematobia irritans* (L.) (Diptera: Muscidae) in Laboratory by Means of Egg and Larva Inoculation

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Abstract

Several studies have required *Haematobia irritans* (L.) raising in laboratory. The present study assessed two methods of inoculating immature forms of *H. irritans* to obtain adults. In 2007, 15 Nellore steers (*Bos indicus*) (L.) were used for the collection of feces free of anthelmintic treatment and flies to produce for eggs and larva. For method I, 30 eggs were incubated in square filter paper (5 × 5 cm) and deposited on bovine feces (500 g) where they were kept until hatching and spontaneous penetration of larvae (L1) into the fecal mass. After 24 h, eggs were analyzed under a stereoscope microscope (40×) for the number of larvae that instinctively penetrated the feces. In method II, larvae were obtained only by natural egg hatching. At birth, 30 larvae were collected and individually inoculated, directly onto the fecal plate by employing a moistened brush. The tests were carried out at controlled temperature (28°C ± 2°C) and saturated humidity (80%) until the emergence of flies with both methods. The number of emerged flies was considered in the result. Using method I, 276 (76.7%) flies emerged from 360 inoculated eggs, while using method II, 283 (78.6%) flies emerged from 360 inoculated larvae. There was no significant difference (P = 0.7821) between methods for the number of flies; however, the proportion between males and females by means of larva inoculation was different from 1:1 (P = 0.0146). Results indicated that both methods led to a satisfactory production of flies and egg inoculation provided an easier establishment.

Keywords

Haematobia irritans, Horn Fly, Hatched Egg, Methods, Breeding

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1. Introduction

Establishment of colonies of the horn fly in laboratory has been long reported [1]-[3].

Insects, such as *Haematobia irritans* L., depend on several abiotic and biotic factors during their short life cycle [4] [5]. Studies have shown the influence of temperature on ovarian development and egg laying [6] [7], as well as the interference of physical and nutritional properties of bovine feces on egg production [8] [9] and larval growth [10]-[12].

The laboratory colony of the horn fly is still an important resource for research and the mass rearing methods published earlier have been improved significantly.

Most data about the first life stages of Diptera result from *in vitro* studies. Evaluations of the effect of various potential field mortalities factors were studied [4]. A colony of the buffalo fly was formed for enhanced studies of its biology and its control [13]. Current changes at the maintenance procedures (blood), and rearing techniques were reflected in an increase in the efficiency of egg and pupal production [14]. Laboratory method adapted for rearing horn fly was used for avaluation of the egg hatch percentage of *H. irritans* [15], aimed at raising flies in laboratory [16]-[18].

Commonly described methodologies for obtaining flies in laboratory are manual deposition of eggs onto fecal [9] [11], fresh [6] [19] and lyophilized mass [20]; egg laying by pregnant females directly onto the feces in laboratory [13]; egg storage in pieces of “organza” fabric [16] or filter paper [4] [5] [8] followed by deposition onto fecal plates.

The present study aimed to evaluate methods for *H. irritans* egg and larva inoculation into bovine feces in order to obtain flies in laboratory.

2. Materials and Methods

In 2007, a batch of 15 male cattle was selected. They were Nellore steers (*Bos indicus*) (L.) aged 30 months, without previous endectocide treatment, and were kept in a paddock covered *Brachiaria decumbens* (Stapf). Flies were obtained by putting an entomological net over *B. indicus*. Immediately after extraction feces were collected in a plastic bucket (20 L) protected from insect contamination. Such material was transported and kept under refrigeration until two h use.

The captured flies were placed in a “tapeware” plastic box (50 cm × 40 cm × 45 cm) for laying on moistened filter paper. All tests were conducted in triplicates of fecal plates, of around 500 g, mounted on aluminum dishes with the bottom covered by a soil layer of 1.5 cm thickness.

The performed inocula accounted for 720, 360 *H. irritans* eggs in method I and 360 *H. irritans* larvae (L1) in method II. In the first method, 30 eggs were counted and grouped on square filter paper (5.0 × 5.0 cm) placed on the bovine feces and kept at 28°C ± 2°C for 24 hours for egg hatching and spontaneous penetration of larvae into the feces. Following this period, the filter paper was then removed from the fecal plate and analyzed under stereoscope microscope (40×) to learn the number of larvae that instinctively penetrated the feces. In method II, the incubated eggs were monitored until hatching. At birth, 30 larvae were counted, randomly collected with the aid of a moistened brush, and individually inoculated directly into the fecal plates. Then, the tests for methods I and II were kept under controlled temperature and saturated humidity until the emergence of adult forms. Following emerging, flies were retained in the “organza” fabric which involved the aluminum dishes used in tests.

After birth, flies were counted under a microscope country stereoscope 40× (Nikon®) and stored in a freezer (08°C) for conservation and sexual differentiation.

The number of emerged flies in for each method was considered the experiment result. A descriptive analysis was done including percentage calculation, mean number of hatched flies, standard deviation for methods I and II, and use of t test to compare the number of hatched flies. Chi-square test was done to verify whether the proportion of males and females was 1:1 in each. Statistical analysis was done by using SAS software, version 9.2, and the adopted significance level was 5%.

3. Results

Using method I, 95% (360/342) eggs/larvae hatched, showing minimum mean of 83.3% and maximum mean of 100%. Of 342 larvae, 76.7% (276) flies emerged, with minimum mean of 40% (12) for test 8 and maximum mean of 100% (30) in for test 2.

Using method II, of 360 inoculated larvae, 78.6% (283) flies emerged, showing minimum mean of 63.3% (19) in for test 8 and maximum mean of 96.7% (29) in for tests 2, 4 and 10.

The statistical analysis of the number of flies obtained by using the methods of egg inoculation and larva inoculation did not show significant difference ($P = 0.7821$) (**Table 1**).

Of 559 flies obtained with both methods, 388 were randomly sampled, to verify the proportion of born males and females 121 were from method I and 267 from method II. In method I, there were 53.7% (65) females and 46.3% (56) males. In method II, there were 66.7% (178) females and 33.3% (89) males (**Table 2**).

The male/female proportion was equal to 1:1 ($P = 0.4133$) for flies born by means of egg inoculation, and different from 1:1 ($P < 0.0001$) for flies born means of larva inoculation. There was a significant difference ($P = 0.0146$) for female proportion between methods.

4. Discussion and Conclusions

Several studies have used inocula of immature forms of flies to establish colonies in laboratory [2] [14] [16]. The production of such Diptera *in vitro* was essential for tests of susceptibility to chemical agents [18] and for the elucidation of their biology [4] [15] [21]. For these studies, eggs have been preferred [11] [16] [19], compared to larvae [22]; although the method used in certain studies is not always reported, differences in procedures could be noticed.

Inoculation can be performed by accomplished depositing the egg on a culture medium [11], or on bovine feces [16] [20] or even naturally by the laying of eggs by females kept in laboratory. The use of a vehicle such as a piece of fabric or filter paper can make easier facilitating the deposition of a larger number of eggs at once on the fecal mass [4] [13].

The factors that influenced these authors in the choice of methods of inoculation of immature fly in the laboratory are unknown. It is believed that the preference would be primarily related to: the purpose of the study, the number of tests, and the difficulty/ease of obtaining biological material or simply by following a methodology already used.

Similarly to Valiela [4], Lima *et al.* [15] [18] adopted a method that allowed egg to hatch on the fecal mass, facilitating the immediate penetration of recently born larvae. According to Anderson [13], this procedure impaired the verification of fertility; however, Valiela [4] and Lima [15] stated that it favored the observation of incubated eggs. The method of inoculation of eggs allowed some facilities: gather the eggs used in the tests earlier; work with quantities that can vary from little or too many eggs; inspect at any time the eggs hatched, have no direct contact with the larva 1, avoiding possible injury during the transfer procedure to the culture medium (cattle dung). In the present study, the methodology of egg inoculation was similar to that used by Lima *et al.* [15] [18]. However, the authors did not bother to evaluate the characteristics of the method. The data analyzed in this work demonstrate the effectiveness of the methods, especially the advantages of each one for future studies.

This strategy allowed the material handling by removing the filter paper containing the eggs, which facilitated the counting of incubated eggs/hatched larvae using method I and was essential to learn the number of penetrated larvae and subsequently emerged flies. Egg inoculation of eggs evidenced the advantage of not having to wait for the exact time of hatching, since when recently hatched larvae left the eggs, recently hatched larvae moved rapidly towards to the fecal mass and instinctively penetrated it.

Differently from the other method I, larva inoculation requires a previous birth monitoring. At birth, larvae emerged simultaneously, requiring greater ability to collect and inoculate the appropriate quantity of individuals in a timely manner. These features made the work difficult; however, this method II allowed simultaneously learning of the exact number of larvae that introduced the feces. Similarly, Smith [22] worked with larvae that were inoculated in artificial food. According to Tomas and Davis [11], the observation of the activities of larvae in the culture medium led to successful fly production; however, they used the egg as inoculum.

Although different studies have quantified the proportion of eggs needed to obtain flies [11] [16] and others have described the use of larvae for inoculation [22], quantitative or even comparative data for both methodologies have not been shown yet.

In the present study, the difference between methods was associated with the advantages of the procedure. There was no significant difference ($P = 0.7821$), regardless of the fly development stage for inoculation (**Table 1**). However, the proportion of female flies born by using the method of larva inoculation was higher than that of males (**Table 2**). This result must be associated with the procedure of larva collection, hypothetically suggesting that “female” larvae present greater mobility than males do and therefore were unwittingly collected first.

Table 1. Analyses of emergence of horn flies, *Haematobia irritans* (L.) by two methods, eggs (method I) and larva (method II) inoculation.

Tests	Method I		Method II	
	Emerged flies	% Flies	Emerged flies	% Flies
1	28	93.3	25	83.3
2	30	100.0	29	96.7
3	23	76.7	24	80.0
4	28	93.3	29	96.7
5	27	90.0	21	70.0
6	27	90.0	20	66.7
7	24	80.0	20	66.7
8	12	40.0	19	63.3
9	27	90.0	23	76.7
10	16	53.3	29	96.7
11	21	70.0	23	76.7
12	13	43.3	21	70.0
Mean	23.0	76.7	23.6	78.6
Standard deviation	6.2		3.7	

P = 0.7821.

Table 2. Analyses of sex ratio of emerged horn flies, *Haematobia irritans* (L.) by two methods, eggs (method I) and larva (method II) inoculation.

Sex	Method I		Method II	
	n	%	n	%
Female	65	53.7	178	66.67
Male	56	46.3	89	33.33
Total	121	100.00	267	100.00

Results indicated a production of 76.7% *H. irritans* (L.) flies through egg inoculation and 78.6% through larva inoculation under conditions of controlled temperature (28°C) and humidity (80%). However, further studies are needed to identify the causes involved in the increased proportion of female flies obtained through larva inoculation.

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