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EXPERIMENTAL CHEMOTHERAPY OF DERMATOBIOSIS IN LABORATORY ANIMALS

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SUMMARY

The unprecedented fact of accomplishing the infection of small size animals (rats, hamsters and mice) with dermatobiosis and their maintenance in laboratory has opened a way to carry out routine experimental chemotherapy. The rat has been chosen as the suitable animal since besides showing maximum morbidity (100%) it has not reached a mortality rate superior to 0.8%. The Authors discuss the validity of screening new chemical compounds using these animals and point out the new possibilities of study especially on the fields of immunology, biology and pathogeny of dermatobiosis, due to the fact that it is possible to infect and maintain small animals in laboratory.

INTRODUCTION

Dermatobiosis is a parasitosis which has been the cause of great concern to many researchers for centuries. GUIMARÃES & PAPAVERO [8], reported that missionaries, physicians and naturalists were concerned about this parasitic problem. RAFAEL MORALES [13], a medical student from Guatemala, found in 1911 that the eggs of *Dermatobia hominis* were carried by mosquitoes. He has also evidenced experimentally that dermatobiosis was provoked by infective larvae originating from those eggs.

In Brazil this parasitosis besides affecting man, leading even to lethal cases (ROSSI & ZUCOLOTO [21]), causes serious economic losses to the country particularly in the field of hide and leather exports. This is due to the fact that this parasite in its larval form may produces heavy damage to the animal's skin especially to cattle.

So far there was no method available that could be easily performed in the laboratory, particularly in the field of experimental chemotherapy.

In this paper the authors describe the maintenance of the life cycle of *D. hominis* under laboratory conditions as well as the accomplishment of the infection of small size animals. This will provide means for investigating the host-parasite relationship, screenings of botcides and carrying out other studies on the field of biology and immunology of this parasitosis.

MATERIALS AND METHODS

a) *Obtaining bots* — The bots used in this experiment were obtained from two different sources: I — Bovine slaughterhouse; II — animals experimentally infected and kept in the animal house.

I — *Bovine slaughterhouse* — Bots which dropped out from the slaughtered animals

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were randomly collected every day. The quantities ranged from 95 to 120 bots. These were stored in a plastic box (24 × 12 × 6 cm, length, width and height, respectively) containing a layer of dry pinewood sawdust *(Araucaria)* of approximately 5 cm in depth. This container was maintained in a humid cabinet (3 × 2 × 4 m — length, width and height, respectively). Lighting was provided by four 40 W fluorescent bulbs fixed on the ceiling of the cabinet. The temperature was maintained constantly at 28°C and the relative humidity controlled at between 70 to 95%. In 20 experiments 2,396 larvae were implanted.

II — *Animals experimentally infected and kept in the animal house* — Bots which fell down from the experimentally infected animals were collected daily in the morning. These bots were stored in the same way as those from the slaughterhouse. In 20 experiments 1,898 larvae were implanted.

b) *Collection of pupae* — Ten days after the seeding the bots that did not transform into pupae were discarded. The pupae already formed (Fig. 1-B) were carefully selected and semi-buried into a sand layer of 3 cm in depth, inside a container, which in turn was placed inside a cage (100 × 60 × 60 cm, length, width and height, respectively). The cages used in this experiment were made of nylon screen (25 mesh/cm^2).

c) *Obtaining of Dermatobia hominis* — 20-40 days after the larvae were implanted the cage containing the pupae was daily examined. The adult flies were collected and transferred to another cage (25 × 25 × 25 cm). They were left undisturbed for 24 hours for mating. Lighting was provided by a 100 W bulb installed at a distance of 20 cm from the cage. Fourty days later the empty puparies (Fig. 1-D) were counted, thus providing the amount of hatched *Dermatobia* in relation to the amount of formed pupae.

d) *Establishment and maintenance of a stock of Musca domestica* — The rearing of *Musca domestica* was based on the method described by ASHBY [1]. Two hundred houseflies were field collected. The captured flies were placed in a cage (45 × 30 × 30 cm, length, width and height, respectively) which was maintained in a humid chamber. Three Petri dishes were placed inside the cage: one with sugar, another one with a cotton pad soaked in water and a third one containing a cotton pad soaked in a mixture of equal parts of powdered milk and water, where the oviposition took place. The eggs were collected daily through the following technique: the cotton pad was removed from the dish by means of a rat tooth forceps. It was gently shaked several times over a beaker containing warm water (25°C). The eggs which dropped from the cotton pad consequently submerged. The supernatant was discarded and the eggs, with quantities ranging from 1,000 to 2,000, were transferred to a glass vial containing adequate culture medium (ASHBY [1]). The vial was closed with cloth mesh secured with elastic band and kept in the laboratory at room temperature. Eight to ten days later the pupae already formed were collected by transferring the culture medium to a beaker containing warm water (25°C). This allowed the pupae to float and they were picked up by means of a metal screen. They were spread on absorbent paper to dry and afterwards were placed in a Petri dish lined with filter paper. The dish was put inside a cage (45 × 30 × 30 cm, length, width and height, respectively) which was maintained in the humid chamber. After two to three days when the majority of the flies had emerged, two dishes were placed inside the cage, one with sugar and one containing a cotton pad permanently soaked with water.

e) *Obtaining of infective larvae of D. hominis* — *Musca domestica* specimens were utilized for that purpose. Four experimental groups were set up. Each group was made up of 200 houseflies and a variable number of *D. hominis*. The first group consisted of 1-10 dermatobiae and the other three groups of 11-20, 21-30 and finally 31-40. The houseflies were left in a cage (45 × 30 × 30 cm, length, width and height, respectively) together with the *D. hominis*. The cage was maintained in a humid chamber. The houseflies were individually examined and those with egglaying (Fig. 1-F) were set apart and placed in a

smaller cage (15 × 15 × 15 cm), containing sugar and water. This cage was placed into the larger one (45 × 30 × 30 cm) and maintained in a humid chamber. Six to eight days later the domestic flies were mechanically sacrificed and the eggs which dropped out were put on the palm of the hand. The larvae which emerged 30 to 45 seconds later were collected and placed on the animal's skin by means of a fine brush (Fig. 1-G).

f) *Animal infection* — 340 Wistar rats (100 g), 30 *Mesocricetus auratus* hamsters (60 to 80 g), 30 mice randomly bred (25 g) and two calves were infected with *D. hominis*. The rodents were anesthetized and a proportional amount of *D. hominis* infective larvae were inoculated. Rats and hamsters were infected with four larvae and the mice with two of them. The calves received 1,500 larvae. These were counted still inside the egg under a dissecting microscope. The larvaed eggs were put on the dorsum of the calves. The larvae immediately started moving and soon penetrated the animal's skin. The rodents (rats, hamsters and mice) were intraperitoneally anesthetized with INOVAL (Johnson & Johnson) at the dosage of 0.003 ml/g body weight and the calves via intramuscular with ROMPUN (Bayer) at the dosage of 0.01 ml/kg body weight. Thus 475 rats, 30 hamsters, 30 mice and two calves were experimentally infected.

g) *Treatment* — Among 475 rats infected, 340 (Table IV) were selected in order to observe the infection behaviour. The other 135 were set apart for studies concerning treatment with a phosphorous drug (0-0-dimetil-2–2–2-trichlore-1-hydroxietil-phosphorous), Dipterex, with known botcide activity. Of the 135 rats, 33 were left untreated serving as control and the other 102 were divided into two groups. The animals from the first group (63 rats) were treated percutaneously and the ones from the second group (39) were treated *per os*. The rats were treated on the 15th day after the infection with single doses of 50, 100, 150 and 300 mg/kg of the phosphorous drug. More detailed information is contained in Tables V and VI.

Cure Control — Two, five and fifteen days after treatment, the animals were mechanically sacrificed and the bots were examined and classified.

h) *Classification of the larval stages* — The classification of the larval stages was made according to BORJA [7], taking into account: a) color of the spines; b) opening of the respiratory spiracle; c) distribution of the spines along the abdominal segments.

TABLE I

Results obtained in the laboratory on pupation and hatching of *Dermatobia hominis* fly (70-95% r.h. and 28ºC)

Source of larvae	No. of experiments	Implanted larvae			Total No. of pupae	Emerged flies *	
		No.	Formed pupae				
			No.	%		No.	%
Slaughterhouse	20	2,396	2.004	83.6	2.004	1,272	63.4
Animal House	20	1,898	1,670	87.9	1,670	1,186	71.0

* 24-40 day period after implantation of the larvae

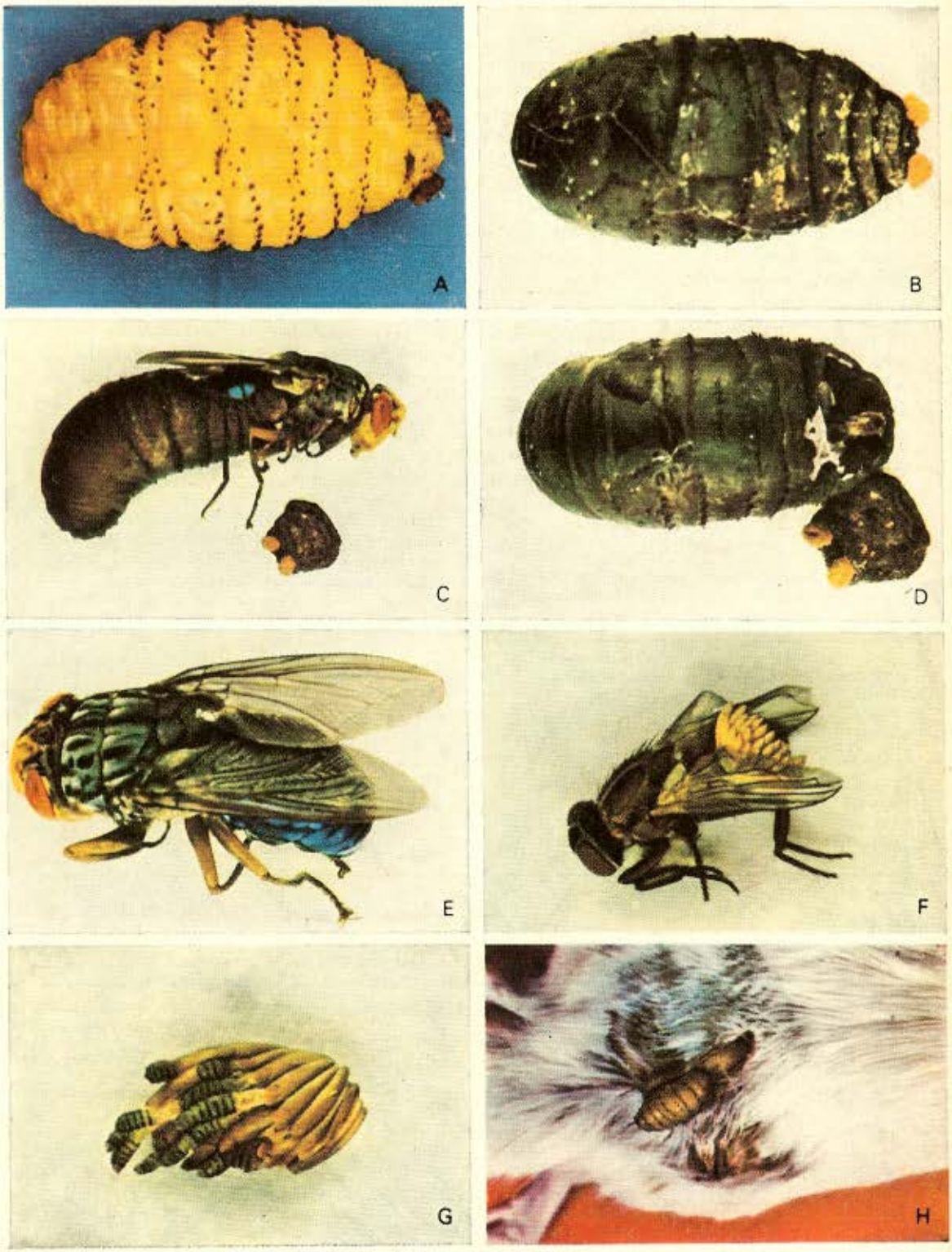

Fig. 1 — Life cycle of *Dermatobia hominis*. A) Bot, larval form; B) Formed puparium; C) Hatching of *D. hominis* fly; D) Empty puparium; E) Adult fly of *D. hominis;* F) *Musca domestica* with eggs of *D. hominis;* G) Infective larval forms of *D. hominis;* H) Rat experimentally infected with *D. hominis*.

RESULTS

I — *Pupation and hatching of D. hominis* — As can be observed by the results stated on Table I, of the 2.396 larvae implanted, proceeding from the slaughterhouse, 2.004 gave origin to pupae (83.6%) and of the 1,898 proceeding from the animal house 1,670 pupated (87.9%). The rates of hatched flies proceeding from the slaughterhouse and from the animal house were 63.4 and 71.0% respectively.

II — *Egglaying of D. hominis on Musca domestica* — The number of *Musca domestica* with oviposition was larger on the group where the amounts of dermatobia were increased. According to the results presented on Table II, the amounts of 1 to 10, 11 to 20, 21 to 30 and 31 to 40 *Dermatobia*, have supplied 99, 170, 340 and 392 respectively, houseflies with egglaying.

TABLE II

Number of *Dermatobia hominis* egglaying on constant number (200) of *Musca domestica* (70-95% r.h. and 28ºC)

No. of *Dermatobia*	No. of experiments	*Musca domestica* with egglaying	
		Total Number	Mean number per experiment
1-10	10	99	9.9
11-20	10	170	17.0
21-30	10	340	34.0
31-40	10	392	39.2

III — *Fertility of Dermatobia hominis eggs* — Among 1,697 eggs of *D. hominis* deposited on houseflies, 818 originated infective larvae, thus resulting in a percentage of 48.2% of eggs hatched. More detailed information is contained in Table III.

IV — *Susceptibility of the rodents to the infection* — All of the rats (340), hamsters (30) and mice (30) submitted to infection with *D. hominis* larvae were successfully infected thus resulting in a 100% infection rate. The mortality of these rodents varied in accordance with the species. Of the 30 mice, 30 hamsters and 340 rats infected, 23, 10 and 3 respectively did not resist the infection (Table IV).

TABLE III

Results obtained on studies concerning the fertility of *Dermatobia hominis* eggs deposited on *Musca domestica* (70-95% r.h. and 28ºC)

No. of *M. domestica* with *Dermatobia* eggs	*Dermatobia* eggs on *Musca domestica*			
	Deposited		Hatched	
	No.	Mean number per *Musca*	No.	%
50	1,697	33.94	818	48.20

TABLE IV

Results obtained on infection and mortality of rats, hamsters and mice experimentally infected with *Dermatobia hominis* larvae

Group	No. of larvae per animal	Animals submitted to infection					
		Rodents	No.	Infected		Died	
				No.	%	No.	%
A	4	Rats	340	340	100	3	0.8
B	4	Hamsters	30	30	100	10	33.3
C	2	Mice	30	30	100	23	76.6

TABLE V

Results obtained in rats infected with 4 larvae of *Dermatobia hominis* and treated percutaneously on the 15th day after infection with different doses of a drug with known action against dermatobiosis

Drug	Dose mg/kg × 1	No. of rats	Autopsy of rats			
			Days after treatment	Rats with bots		% of cured rates
				Alive	Dead	
Dipterex	300	4	2	0	4/4	100
		4	5	0	4/4	100
		4	15	0	4/4	100
	150	4	2	0	4/4	100
		4	5	1/4	3/4	75
		4	15	1/3 **	2/3	66
	100	9	2	1/9	8/9	88
		9	5	3/9	6/9	66
		9	15	3/9	6/9	66
	50	4	2	3/4	1/4	25
		4	5	3/4	1/4	25
		4	15	3/4	1/4	25
Untreated Control	—	5	17 *	5/5	0/5	0
		5	20 *	5/5	0/5	0
		5	30 *	5/5	0/5	0

* Days after infection ** One rat died

TABLE VI

Results obtained in rats infected with 4 larvae of *Dermatobia hominis* and treated orally on the 15th day after the infection with different doses of a drug with known action against dermatobiosis

Drug	Dose mg/kg × 1	No. of rats	Autopsy of rats			
			Days after treatment	No. of rats with bots		% of cured rats
				Alive	Dead	
Dipterex	300	4	2	0/4	4/4	100
		4	5	0/4	4/4	100
		4	15	0/4	4/4	100
	150	4	2	1/4	3/4	75
		4	5	0/4	4/4	100
		4	15	1/4	3/4	75
	50	5	2	3/5	2/5	40
		5	5	1/5	4/5	80
		5	15	2/5	3/5	60
Untreated Control	—	6	17 *	6/6	0/6	0
		6	20 *	6/6	0/6	0
		6	30 *	6/6	0/6	0

* Days after the infection

V — *Treatment* — The cure rates in the groups of rats treated with 300 mg/kg body weight of dipterex were 100%. With lower doses (50, 100 and 150 mg/kg) given either orally or percutaneously, the cure rates ranged from 25 to 100%. More detailed information is contained on Tables V and VI.

TABLE VII

Results obtained on spontaneous elimination of bots originating from calves infected with 1,500 larvae of *D. hominis*

Calf Number	Bot Fall	
	Week after the infection	Quantity
1	5th	447
	6th	408
	7th	37
2	5th	287
	6th	99
	7th	1

VI — *Elimination of bots* — The elimination of bots from the calves experimentally infected took place during the 5th week after the infection. As can be observed by the results stated on Table VII, the higher number of eliminated bots, either for calf number one (447 and 408) and for calf number two (287 and 99) occurred during the 5th and 6th weeks after the infection, decreasing in the 7th week (37 and 1).

DISCUSSION

The life cycle of *D. hominis*, from obtaining pupae to accomplishing the infective larval forms, has been studied by many scientists. URBINA [25], KOONEY & BANEGAS [11] have pointed out the importance of the soil humidity in the emergence of adult forms. BANEGAS et al. [3] and BORJA [7], have used humid soil and sand for obtaining pupae and adults. ZELEDON [27], has used different means (wood straw, humid and dry soil, absorbent paper, etc.) and come to the conclusion that any absorbent material in suitable for this purpose. ZELEDON [27], BORJA [7] and BANEGAS et al. [3] found that the temperature at 24 to 28°C and the relative humidity held between 70-90% were the ideal environmental conditions for the formation of pupae and for fly emergence.

For this reason, in the present work the authors have used pinewood sawdust *(Araucaria)* for implanting larvae and the temperature was maintained at 28°C and the relative humidity ranging from 70 to 95%.

Some researchers, among them PEÑA et al. [17], BATES [4], PINTO [18], ZELEDON [27] and NEEL et al. [14], captured in the field some insects, such as *Stomoxys calcitrans, Sarcopromusca serratus, Neivanya lutzi, Psorophora ferox* and *Aedes serratus*, with eggs of *Dermatobia hominis*. NEEL et al. [14] and ARTIGAS & SERRA [2], have performed a bibliographic survey concerning the most common vectors of *Dermatobia hominis*, which belong the following families: *Culicidae, Anthomylidae, Simulidae, Muscidae, Calliphoridae* and *Tabanidae*. In the life cycle of *D. hominis* completed in laboratory we have utilized *Musca domestica* as a vector, because it is an easily reared insect besides being suitable for egglaying of *D. hominis*. As a matter of fact *Musca domestica* has been used previously with success by BORJA [7], BANEGAS et al. [3], and ZELEDON [27].

Some Authors such as BORJA [7], ZELEDON [27], KOONE & BANEGAS [11], NEIVA & GOMES [16], VIVAS - BERTHIER [26], TOLEDO [23], JAMES [10], SOULSBY [22] and BANEGAS et al. [3], reported that the incubation period of the eggs ranged from 5 to 15 days. They still believe that the incubation period as well as the maintenance of the infective larvae of *Dermatobia hominis* are closely related to the environment humidity and temperature. Of the 1,697 eggs (Table III) of *D. hominis* deposited on *M. domestica* and maintained at the temperature of 28°C, and the relative humidity from 70 to 95%, 818 produced infective larvae 6 days after the egglaying. These figures can be considered quite satisfactory if we take into consideration that BORJA [7] found that 25 to 50% of the *Dermatobia* eggs are infertile.

Some medium size animals such as dogs, goats and rabbits had been experimentally infected in the laboratory or encountered naturally infected by many researchers, among them NEIVA [15], RIBEIRO [20], TOLEDO & SAUER [24], BORJA [7], ZELEDON [27], PINTO [19] and NEIVA & GOMES [16]. However, these animals are not suitable for laboratory experiments especially those concerning experimental chemotherapy when large amounts of new compounds, which generally are synthesized in small quantities (mgs), are involved. For this reason the authors decided to look for an animal with the following characteristics: small size, high fecundity, easy handling in the laboratory and low cost, thus enabling the performance of hundreds of tests with new chemotherapic compounds in the field of dermatobiosis.

Table IV indicates that virtually all hamsters, rats and mice which were submitted to infection became infected. However, even though hamsters and mice became easily infected they are not suitable for laboratory tests due to the high mortality rates, which were 33.3 and 76.6%, respectively, unlike the rats which presented a quite insignificant mortality rate (0.8%).

In order to obtain the complete life cycle of *D. hominis* in laboratory, there are still some steps to be overcome. It will be necessary to easily obtain large quantities of the larval forms (bots). There are currently two sources of bots: the bovine slaughterhouse and those obtained from the bovines experimentally infected with 1,500 larvae and stabled in the animal house. This number of infective larvae, previously established by BERG [5], provides large amounts of bots (Table VII). Further studies are being conducted by the authors with the aim of substituting the bovine for the rat, as a probable source of bots. The first results are encouraging and we are confident that in the near future the full life cycle of *D. hominis* will be easily attained in the laboratory.

Recent evidence (BOLLE [6] and MENDEZ [12]) has shown that dipterex is a drug with therapeutic action against dermatobiosis in bovines. For this reason different doses of this drug were administered to rats experimentally infected with *D. hominis* in order to find out if the therapeutic response will be the same as in bovines. Tables V and VI indicate that two days after treatment the bots were already killed thus showing that dipterex had displayed action against the bots of these rodents. These results suggest that the rat is a suitable animal especially in the preliminary screenings when new compounds synthesized especially for that purpose, have to be tested.

The Authors believe that the use of "in vivo" methods of screenings are more advantageous than those "in vitro" (IWUALA [9]) particularly when the compounds are insoluble.

The results obtained in the present study lead the Authors to believe that it has greatly contributed to the field of study of dermatobiosis. Furthermore providing facilities for studies of new compounds in the laboratory it may be a valuable tool for further research on the fields of biology, immunology and pathology of this parasitosis.

RESUMO

Quimioterapia experimental da dermatobiose em animais de laboratório

O fato pioneiro de se conseguir infectar e manter animais de pequeno porte (ratos, hamsters e camundongos) com a dermatobiose, abriu perspectivas para a realização rotineira da quimioterapia experimental. O rato se apresenta como animal de escolha, pois, além de revelar máxima morbidade (100%), não atingiu um índice de mortalidade superior a 0,8%.

Os Autores discutem a validade dos testes de novos compostos quando se utilizam estes animais e destacam os horizontes que se abrem, principalmente no campo da imunologia, biologia e patogenia da dermatobiose, visto que já se pode facilmente manter e infectar animais de pequeno porte em laboratório.

REFERENCES

1. ASHBY, G. J. — The House Fly — *in* HUME, C. W. — The UFAW *Handbook on the care and management of laboratory animals.* Edited by UFAW. London, 1972.

CHAIA, G.; BORJA, G. E. M.; CHIARI, L.; SANTOS, C. N. dos & ABREU, R. T. L. — Experimental chemotherapy of dermatobiosis in laboratory animals. *Rev. Inst. Med. trop. São Paulo* **17**: 298-306, 1975.

2. ARTIGAS, P. de T. & SERRA, R. G. — Portadores de ovos de *Dermatobia hominis* (L. Jr., 1781). Atualização da lista de foréticos com a enumeração de novos agentes transmissores do "berne". *Ciência e Cultura* (Brasil) — *in* BORJA, G. M. — *Estudios sobre la biología, morfología y esterilización del tórsalo, Dermatobia hominis* (L. Jr.). [*Tesis*]. Magister Scientiae, Inst. interam. de Ciências Agrícolas de la O.E.A., 63 pp, Turrialba, Costa Rica, 1966.

3. BANEGAS, A. D.; MOURIER, H. & GRAHAN, O. H. — Laboratory colonization of *Dermatobia hominis* (Diptera: cuterebridae). Entomological Society of America. *Annals* **60**:511-513, 1967.

4. BATES, M. — Mosquitoes as vectors of *Dermatobia* in eastern Colombia. Entomological Society of America. *Annals* **36**:24, 1943.

5. BERG, G. H. — El tórsalo. *Agricultura en El Salvador* **4**:3-5, 1963.

6. BOLLE, W. R. — Neue Wege zur Bekaempfung der *Dermatobia hominis* mit Neguvon und Asuntol. *Veter. Med. Nachr.* **4**: 193-206, 1858 — *in* GUIMARÃES, J. H. & PAPAVERO, N. — A tentative annotated bibliography of *Dermatobia hominis* (Linnaeus Jr., 1781) (Diptera: cuterebridae). *Arq. Zool.* **14**:223-294, 1966.

7. BORJA, G. M. — *Estudios sobre la biología, morfología y esterilización del tórsalo, Dermatobia hominis* (L. Jr.). [*Tesis*]. Magister Scientiae, Inst. Inter-Amer. de Ciências Agrícolas de la O.E.A. 63 pp, Turrialba, Costa Rica, 1966.

8. GUIMARÃES, J. H. & PAPAVERO, N. — A tentative annotated bibliography of *Dermatobia hominis* (L. Jr., 1781) (Diptera: cuterebridae). *Arq. Zool.* **14**:223-294, 1966.

9. IWUALA, O. M. — Standardization of a resistance test for myasis producing larvae. *Trans. Royal Soc. Trop. Med. & Hyg.* **68**: 13-14, 1974.

10. JAMES, M. T. — The flies that cause Myasis in man. U. S. Department of agriculture; Miscellaneous Publication — No. 631, 1947.

11. KOONE, H. D. & BANEGAS, A. D. — Biology and control of *Dermatobia hominis* in Honduras. *J. Kansas Entomol. Soc.* **32**: 100-108, 1959.

12. MENDEZ, M. M. M. — El Neguvon en el tratamiento de la *Dermatobia hominis*. *Rev. Med. Vet. & Parasitol.* (Venezuela) **1-8**: 111-124, 1959-1960.

13. MORALES, R. — Citado *in* PESSOA, S. B. — *Parasitologia Médica*. 8.ª edição. Rio de Janeiro, Edit. Guanabara Koogan S.A., 1972.

14. NEEL, W. W.; URBINA, O.; VIALE, E. & ALBA, J. — Ciclo biológico del tórsalo *(Dermatobia hominis* L. Jr.) en Turrialba, Costa Rica. *Turrialba* **5**:91-104, 1955.

15. NEIVA, A. — Contribuição ao estudo da biologia da *Dermatobia cyaniventris* Macq. *Brasil Médico* (Rio de Janeiro) **22**:311-312, 1908 — *in* GUIMARÃES, J. H. & PAPAVERO, N. — A tentative annotated bibliography of *Dermatobia hominis* (Linnaeus Jr., 1781) (Diptera: cuterebridae). *Arq. Zool.* **14**:223-296, 1966.

16. NEIVA, A. & GOMES, J. F. — Biologia da mosca do berne *(Dermatobia hominis)* observada em todas as suas fases. *Ann. Paul. Med. e Cir.* **8**:197-209, 1917.

17. PEÑA CHAVARRIA, A. & KUMM, H. W. — Algunas consideraciones generales sobre la miasis cutánea (tórsalo) en Costa Rica. *Rev. Med.* (Costa Rica) **3**:635-641, 1939.

18. PINTO, C. — Tratado de Parasitologia — Arthropodes, Oestrideos. Cap. XIV. Editora Pimenta de Melo & Cia., 451-468, 1930.

19. PINTO, C. — Zoo-parasitos de interesse médico e veterinário. 2.ª edição. Rio de Janeiro, Edit. Scientifica, Cap. 17, 1945.

20. RIBEIRO, R. — O berne. *Correio Agrícola* (Brasil) **4**:257-260, 1926.

21. ROSSI, A. M. & ZUCOLOTO, S. — Fatal cerebral myasis caused by the tropical warble fly *Dermatobia hominis*. *Amer. J. Trop. Med. & Hyg.* **22**:267-269, 1973.

22. SOULSBY, E. J. L. — Helminths, Arthropods and Protozoa of domesticated animals. Sixth Edition. London, 1968.

23. TOLEDO, A. A. — Notas sobre a biologia e controle do berne. *Biológico* (Brasil) **14**: 264-267, 1948.

24. TOLEDO, A. A. & SAUER, F. H. — Efeito de alguns inseticidas clorados sobre o berne. *Biológico* (Brasil) **16**:25-34, 1950.

25. URBINA, O. — Efecto del tórsalo *(Dermatobia hominis)* (L. Jr., 1781) en la productividad del ganado de carne y algunos aspectos de su infestación. [*Tesis*]. Mag. Agr. Turrialba, Costa Rica, Inst. Interamer. Ciéncias Agrícolas, 1954.

26. VIVAS-BERTHIER, G. — El gusano de monte, de zancudo o de mosquito. *Rev. Med. Vet. & Parasitol.* (Venezuela) **3**:129-152, 1941.

27. ZELEDON, R. — Algunas observaciones sobre la biologia de la *Dermatobia hominis* y el problema del tórsalo en Costa Rica. *Rev. Biol. Trop.* (Costa Rica) **5**:63-75, 1957.

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