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BIOLOGY OF *CUTEREBRA LEPUSCULI* TOWNSEND (DIPTERA: CUTEREBRIDAE) IN COTTONTAIL RABBITS IN IDAHO[1]

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ABSTRACT: *Cuterebra lepusculi* Townsend parasitize cottontail rabbits (*Sylvilagus nuttallii*) in southern Idaho. Peak parasitism was 69% in mid-September. Mean development time in the host was 27 days. The species is univoltine in Idaho. Partially developed larvae were transferred from freshly killed to living hosts and the resulting larvae matured normally. Developing pupae were cooled or warmed to retard or speed development and synchronize adult emergence.

INTRODUCTION

Cuterebra lepusculi Townsend causes cutaneous myiasis in cottontail rabbits in the western United States. Larvae of this species were first reported from New Mexico by Townsend (1892) who provided additional descriptions of adults and larvae (Townsend, 1893) but erroneously identified them as *C. fontinella*. Further observations resulted in the description of a new species, *C. lepusculi* Townsend (Townsend, 1897), but the species was later designated a synonym of *C. princeps* (Townsend, 1917). *Cuterebra lepusculi* is now considered a valid species (Sabrosky, pers. comm.).

Life history data on this species have been almost non-existent until 1977 when a heavily parasitized population of cottontail rabbits was discovered in southwestern Idaho. This paper reports a 4-yr study of *Cuterebra lepusculi* in captive and wild cottontail rabbits.

MATERIALS AND METHODS

Techniques for rearing *Cuterebra* under laboratory conditions have been reported by several workers (Bennett, 1955, 1972; Catts, 1964; Capelle, 1970; Baird, 1971, 1972; Smith, 1973, 1977). *Cuterebra* larvae were collected from live-trapped cottontails and by examining fresh road-kills. Host collections were made year 'round; however, intensive sampling of cottontail populations was conducted from July through October (1978–1980) when at least 30 rabbits were collected each month. Rabbits were collected near Middleton, Canyon County and Rogerson, Twin Falls County in 1977–1980 and Aberdeen, Bingham County in 1979–1980. Black-tailed jackrabbits (*Lepus californicus*) and white-tailed jackrabbits (*L. townsendi*) also were collected and examined for bot fly larvae.

Cottontail rabbits, black-tailed jackrabbits, and domestic rabbits (*Oryctolagus cuniculus*) were maintained in captivity and challenged with experimental doses of infective larvae of *C. lepusculi* introduced via nasal and oral openings.

Mature *Cuterebra* larvae from naturally infected rabbits were allowed to pupate in individual jars of sand. Immature larvae were surgically removed from dead hosts and maintained alive in vertebrate physiological saline for up to 48 hr. The larvae were transferred to living hosts by shaving the host's fur from 4 × 4 cm areas of the dorsum, making a 1 to 3 cm incision through the shaved skin, and implanting live, partially grown larvae beneath the skin. Six cottontails received subcutaneous implants of one to three larvae each.

After pupation, the operculum was carefully removed from each puparium so that pupal development could be observed (Baird, 1972a, 1975). Pupae were maintained in individual petri dishes at room temperature (20–22 C) and 60–75% RH. At termination of diapause, most pupae were contained at room temperature and allowed to develop normally. Slowly developing pupae were maintained at 27–30 C to speed development. Rapidly developing pupae were refrigerated at 4–6 C to retard development. These temperature manipulations helped to synchronize the emergence of a maximal number of flies at a given time.

Representative specimens have been deposited in the U.S. National Parasite Collection (Beltsville, Maryland) as accession no. 77609.

RESULTS

Field studies

The prevalence of *C. lepusculi* in cottontail rabbits is shown in Table 1. No *C. lepusculi* were found on 34 black-tailed or 11 white-tailed jackrabbits sharing the cottontail habitat; however, 9% of the black-tailed jackrabbits were parasitized by *C. ruficrus*. *Cuterebra lepusculi* larvae were taken from three of seven road-

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TABLE 1. Prevalence of *Cuterebra lepusculi* infection in cottontail rabbits in Idaho.

	Prevalence[a] (%)							
	July	Aug	Sept	Oct	Nov	*n*	$\bar{x}$	SD
1977	5.0	43.4	66.7	—	—	73	38.4	31.2
1978[b]	6.7	37.5	71.8	41.9	3.2	156	32.2	28.2
1979	10.0	40.0	72.2	32.3	0	160	30.9	28.2
1980	3.3	36.7	65.6	19.4	3.3	153	25.7	26.2
$\bar{x}$	6.2%	39.4%	69.1%	31.2%	2.2%			
SD	2.9	3.0	3.4	11.3	1.9			

[a] Hosts were recorded as positive if larvae or recently vacated warbles were present.
[b] During 1978–1980, a minimum of 30 rabbits were examined each month.

killed pigmy rabbits (*Sylvilagus idahoensis*) examined in 1979–1980.

On cottontails, the back/rump area was the most common warble site (56%) although larvae were frequently found near the front shoulders (25%) and on the neck (17%). Larvae were found in ventral warbles on 2% of the infected rabbits. Infected rabbits carried from one to 14 larvae with a mean of 3.3 per infected rabbit. Multiple larvae in a single warble were occasionally found.

Morphology and activity of adult flies

Adult *C. lepusculi* resemble *C. jellisoni* and *C. lepivora* in general appearance, however, *C. lepusculi* has a more uniform and consistent spot pattern on the abdomen. The most distinguishing feature is the white pile around the entire edge of the scutellum on *C. lepusculi* which is absent on *C. jellisoni* and *C. lepivora*. The red eye patterns of all three species are alike and quite variable (Catts and Radovsky, 1962; Baird, 1971, 1972). All red color faded within 4 hr after death.

Adult flies mated readily at temperatures of 27–35 C when tethered (Baird, 1971) or tumbled (Smith, 1973) but were difficult to mate at room temperature (20–22 C). The age of mated flies ranged from 2 days with flies maintained at 22 C, to 18 days for flies refrigerated at 4–6 C. Duration of mating for seven mated pairs averaged 3.5 min (range = 1.5–6 min).

Mated females began ovipositing at 5 to 6 days of age, but none laid its full complement of eggs. Total egg counts of five dissected flies ranged from 880 to 1,385 ($\bar{x}$ = 1,286). Egg hatching occurred spontaneously after five days at 27–30 C but was delayed until 12 days at 13–18 C.

Larval development in *Sylvilagus nuttallii*

Larvae were first visible on the 8th post-entry day when they established larval breathing pores. The larvae had molted to second instar when the small skin lesions were first observed. The molt from second to third instar occurred on the 14th day. Larvae matured and exited the host in 25–35 days ($\bar{x}$ = 27.7 days for 129 larvae). However, four larvae in the scrotal area required 35 days to develop and had difficulty exiting the host. Most larvae (64%) developed in warbles on the back and rump; however, warbles were also located on the shoulder area (22%), ventral and inguinal area (9%), and on the head and neck (5%).

Cottontails supported larvae without difficulty at doses of eight to 15 larvae per host in most cases (Table 2). Two rabbits became emaciated and died while carrying seven and eight nearly mature larvae during their second and third infections. However, several hosts supported seven to nine larvae to maturity without apparent difficulty.

Experimental infections in *Lepus* and *Oryctolagus*

Black-tailed jackrabbits and laboratory rabbits were not suitable hosts for rearing *C. lepusculi* (Table 2). Heavy wheezing was noted in all these hosts within 24 hr after larval introduction, and in most cases, partial or complete paralysis of the hind limbs occurred within 48 hr. One laboratory rabbit supported larvae to near maturity (19 days), however, the warbles were poorly formed and the larvae died in situ. No larvae introduced into jackrabbits developed to second instar.

TABLE 2. Experimental infections of *Cuterebra lepusculi* in lagomorph hosts.

Host species	No. of hosts	Larval dosage	% Positive infections	% of larvae reaching maturity	% Host paralysis or mortality
Sylvilagus nuttallii	9	10–12	100	70.3	0
Sylvilagus nuttallii[a]	8	8–15	100	44.8	12
Sylvilagus nuttallii[b]	6	8–10	67	25.0	17
Lepus californicus	5	10	0	0	60
Oryctolagus cuniculus	6	7–10	33	0	83

[a] Hosts had received laboratory-induced infection once previously
[b] Hosts had received laboratory-induced infection twice previously

Surgical transfer of larvae from dead to living hosts

Partially developed larvae were successfully removed from freshly killed cottontails and implanted beneath the skin of living cottontails. Second instars or early third instars were more successfully transferred in this manner. Surgical transfer was successful with nine of 11 larvae in six hosts. Nine larvae completed development and exited the host normally. Normal puparia were formed by all larvae within 24 hr. Three of the nine larvae died after forming a puparium but before pupation, and two died after pupating but before eclosion as adults. Only four completed development as adult flies.

Suturing the incision to retain the larva and reduce the size of the opening resulted in the death of two larvae. The incision scabbed over and the larvae did not maintain breathing pores.

Normal warbles were formed only with the smallest of the second instars. Third instars were in their hosts insufficient time for a warble to form. The incisions warble pores healed rapidly after larval exit, and the hosts recovered uneventfully.

Observations on pupal stage

Mature larvae burrowed into sand or dirt within minutes after exiting the host and formed puparia within 24 hr. Pupation followed 6 days later and pupae entered diapause immediately. Removal of the operculum had no adverse effect on development as long as it was done after pupation had occurred, i.e., 6–7 days post-pupariation.

The development of pupae in advanced stages (post-diapause) was slowed by refrigerating them at 4–6 C. Conversely, rearing less developed pupae at 27–30 C speeded development. These temperature manipulations maximized the number of flies emerging within a short time period and ensured sufficient numbers of adult flies for mating purposes. Only post-diapause pupae were affected by temperature manipulations. As was noted by Baird (1975), cold temperatures did not affect the pupal duration of diapausing pupae. No adverse effects of the temperature manipulations were noted as long as pupae were maintained at room temperature after the head had begun to darken.

The duration of the pupal period for larvae reared from wild hosts was 235 days (208–252) for 15 males and 230 days (191–292) for 14 females. Pupae resulting from laboratory infections developed in 220 days (153–256) for males and 223 (140–281) for females.

DISCUSSION

Myiasis in wild cottontails

The highest prevalence of *C. lepusculi* myiasis occurred in mid-September (Table 1). Year to year variation in prevalence was not significant (ANOVA-arcsin transformation, $F = 0.16$, $P < 0.90$, df = 3,14), however, monthly variation was highly significant ($F = 53.77$, $P < 0.0005$, df = 4,13). This is similar to other studies with *Cuterebra* in cottontail rabbits (Geis, 1957; Haas and Dicke, 1958; Jacobson et al., 1978), although the September peak of 69% in Idaho was considerably higher. Other species of *Cuterebra* are occasionally found in *Sylvilagus nuttallii* in Utah and Idaho, including *C. jellisoni* and *C. ruficrus* both of which are jackrabbit bot flies (Baird, 1971, 1972b). In central Washington, *C. lepivora* was taken from *S. nuttallii* during July and August (unpubl. data).

Development of *C. lepusculi* in lagomorph hosts

Cuterebra lepusculi developed and matured in less time in *S. nuttallii* than other lago-

morph–bot fly relationships thus far recorded. This is evidenced in the early molts to second and third instar at 8 and 14 days respectively, and the total larval development period of 27 days. This compares to *C. jellisoni*: 36 days in jackrabbits and 33 days in cottontails; *C. ruficrus*: 74 days in jackrabbits and 68 days in cottontails (Baird, 1971, 1972b). Jacobson et al. (1978) reported 30–33 days for *C. buccata* in laboratory rabbits. Haas and Dicke (1958) estimated 28 to 32 days for *C. horripilum* (=*C. abdominalis*) in *Sylvilagus*. *Cuterebra lepivora* required 37–43 days to develop in *S. audubonii* (Catts, 1982).

Cuterebra lepusculi developed readily in cottontails but not in jackrabbits or laboratory rabbits. It is surprising that no successful development occurred in the other lagomorphs since other *Cuterebra* species have demonstrated some host crossover ability (Catts, 1965; Baird, 1971, 1972b; Jacobsen et al., 1978). Host specificity is also demonstrated in the high percentage of positive infections in captive cottontails, the high rate of larval completion and the low rate of host mortality (Table 2). Previous tests with *Cuterebra* in laboratory rabbits have given mixed results. Jacobsen et al. (1978), Weisbroth et al. (1973) and Capelle (pers. comm.), with *C. buccata* and *C. ruficrus*, respectively, successfully reared larvae to maturity in *Oryctolagus*. However, results during this study with *C. lepusculi* and those of Ryckman and Lindt (1954) with *C. lepivora*, were negative and caused host mortality.

The reduced larval success in second and third successive infections indicates some level of acquired immunity. However, there was considerable variability in this study, and sample sizes were small. Partial immunity has been reported in previous studies (Weisbroth et al., 1973; Gingrich and Barrett, 1976; Baird, 1979).

Laboratory mating of adults was most successful when room temperatures were 23–35 C. Below 23 C the flies tended to be inactive for their entire adult life, and most mating attempts were unsuccessful. Smith (1973) reported higher mating success of *C. approximata* at 21 C than at lower temperatures.

Egg capacity for *C. lepusculi* females (1,286) was similar to that reported for most other rabbit bot flies, *C. jellisoni* (1,006), *C. buccata* (1,534) but less than *C. ruficrus* (1,727) which is a much larger fly (Baird 1971, 1972b; Jacobson et al., 1978).

Additional techniques in rearing *Cuterebra*

During this study, two additional techniques were tested for assisting research on bot flies: (1) cooling or warming developing pupae to retard or hasten development in order to synchronize emergence of adult flies, and (2) surgical transfer of immature larvae from a dead host to a living host.

In the past, it has been difficult to obtain sufficient numbers of male and female flies at the same time to ensure mating and obtain fertile eggs for laboratory infections. Cooling or warming pupae was very useful in synchronizing adult emergence. This technique should not be confused with earlier attempts to terminate diapause by chilling since the pupae in this study had already resumed development. Temperature was used to manipulate the rate of pupal development, not to terminate pupal diapause.

One difficulty in the study of field-collected *Cuterebra* larvae has been the inability to identify partially developed specimens. Mature larvae usually pupate and emerge as adults which can be identified, but second and third instars are lost when the host dies. Bennett (1955, 1972) first transferred *C. emasculator* larvae from dead to living chipmunks (*Tamias striatus*). More recently, Jacobson et al. (1978, 1981) performed the first successful transplants of *C. buccata* in eastern cottontails (*S. floridanus mallurus*), but were unsuccessful in transplanting *C. emasculator* in gray squirrels (*Sciurus carolinensis*). During this study, experiments in which larvae were transplanted in new hosts further demonstrated that the technique is useful and practical. The technique should be useful in rearing unidentified larvae to maturity for identification and laboratory studies.

Shaving the host's skin was necessary in order to make an incision in the delicate skin of a cottontail. The incision should be only large enough to accommodate the larva. Suturing an oversize incision resulted in larval mortality when the scab formed over the opening.

During this study there was unexplained mortality in the pupal stage even when post-surgery larval development appeared normal. Emerging flies appeared normal in all respects including morphology, activity, and fertility.

Summary of *C. lepusculi* life history

In Idaho, adult flies probably emerge in the wild between mid-June and the first week in

July. Mating and egg laying follows within 7–8 days and the earliest cases of myiasis are found in cottontail rabbits in mid-July. Eggs are probably laid on grass or other objects along rabbit trails and attach and enter the host via oral and nasal openings. About 27 days are required for larval development. As larvae mature, they exit the host and burrow into the soil to pupate and overwinter in a state of diapause. Development resumes in April or May and adult eclosion occurs 30–60 days later. There is one generation per year.

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