The method by which *Cephenemyia trompe* (Modeer) larvae invade reindeer *(Rangifer tarandus)*

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Abstract: Laboratory electrostimulated *C. trompe* (Modeer) females forcefully expelled (sprayed) larvae for 5-20 cm. The watery spray consisted of about 20 tiny droplets containing two to several larvae. Crawling first-instar larvae exhibited negative geotactic and phototropic responses; they were subject to rapid desiccation and became immobile as the tiny droplets dried within a few seconds. When 5-50 larvae from dissected females were dropped in physiological saline onto different areas of the muzzle of restrained reindeer, only larvae placed deep within the nostrils and on the lips crawled out-of- sight down the nostril passage or into the mouth. Drops of larvae placed elswhere quickly desiccated and the larvae became immobile. Larvae deposited by wild females onto a CO_2-baited reindeer model with the muzzle, lips and nostrils coated with insect trapping adhesive all were stuck only along the dorsal lip below the philtrum. All experimental evidence supports a natural *per os* mode of invasion.

Key words: attack, larval invasion, Norway

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Introduction

Speculation on how *Cephenemyia trompe* (Modeer), the larviparous female nasopharyngeal bot fly of reindeer, *Rangifer tarandus*, infects its host has ranged from folklore statements about flies flying into the nostrils to reports of females spraying, squirting or depositing larvae onto the hosts' nose or into the nostrils (Bergman 1917; Natvig, 1917; Hawden, 1927; Grunin, 1957; Espmark, 1967). More recent research on two species of nasopharyngeal bot flies of California blacktailed deer *(Odocoileus hemionus columbianus)* revealed that larvae of *C. apicata* (Bennet & Sabrosky) and *C. jellisoni* (Townsend), when placed experimentally on various parts of the muzzle of deer, always immediately crawled ventrally and entered the mouth of the host (Cogley and Anderson 1981). Subsequent research in California with deer models baited with carbon dioxide revealed that larvae expelled naturally by wild *Cephenemyia* females always were stuck to the lower surface of the muzzle and the lips (Anderson, 1989).

The objective of our research with *C. trompe* was to study how its larvae were expelled and

how they invaded reindeer, and to make comparisons with the other *Cephenemyia* species studied.

Methods of study

The first phase of our research involved laboratory electrostimulation of live *C. trompe* females. The females used in these experiments were host-seeking flies that had been captured in Finnmark, Norway, in CO_2-baited insect flight traps like those used by Anderson and Olkowski (1968) and Anderson and Hoy (1972). Captured females were transferred to glass holding vials ventilated by several small holes in their snap caps. The females were held at about 5° C until used from 2-5 days later.

Females were glued to microscope slides that were positioned so the head and body of an electrostimulated female was aligned at a 60-75° angle from the microscope stage. The reactions of such females could then be observed with a dissecting microscope as they were electrostimulated. As described by Cogley and Anderson (1981), a Grass® S88 electric stimulator adjusted to 40-80 volts (duration of 8 ms, frequency of 50 pulses/sec.) was used to induce females to expel larvae. Females were stimulated along the ventral nerve by touching the exposed leads to the ventral surface of the intersegmental area between the thorax and abdomen.

Groups of about 5-15 expulsed larvae were picked up from uterine fluid on the bristles of a camelhair brush or an insect pin, and their crawling behavior and time to desiccation observed while viewed with a dissecting microscope. To determine the effect of desiccation time on larval recovery, larvae that had dried on brush bristles were placed in physiological saline or human saliva at different intervals after drying. Larval tropisms were determined by holding saline moistened brushes with groups of larvae on them towards a bright light and at different angles, and observing in which direction larvae crawled.

Other larvae were obtained when the uterus of females was dissected in physiological saline. Groups of 25-50 such larvae were pipetted to spot plate depressions and held in the saline for up to 30 minutes.

Semi-tame reindeer females were restrained by hand and in a squeeze box while larvae in drops of physiological saline were experimentally placed onto their muzzle, in and around the nostrils, and on the lips. Groups of about 25-50 larvae were picked up with a 1.5 cc bulbed pipette and delivered to the muzzle of the host 8 times in a single drop of saline. In six other instances just 2-10 larvae were delivered to the hosts'muzzle in a smaller drop of saline. Four times drops of about 25 larvae were placed several cm deep into the nostrils of a reindeer (beyond the fringe of long guard hairs surrounding the entrance). Drops of from 5-25 larvae were placed onto the upper or lower lips of reindeer six times.

Figure 1. Experimental reindeer model exposed in a birch woods habitat. A curved vinyl tube leads from a styrofoam box containing dry ice into the head of the model where sublimated CO_2 was released from the mouth.

In 1987 an experimental reindeer model (Fig. 1.), similar to deer models previously used in California (Anderson, 1989), was exposed in

both birch woods and highland plains habitats. The reindeer model consisted of a taxidermically-prepared head attached to a half-meter board mounted on a tree stump or wood frame. The body of the model was formed by placing reindeer hides over cardboard boxes and the frame. Dry ice was used as a source of CO_2 About 2 kg of dry ice was placed in a styrofoam insulted box that rested on the ground below the model's head. One end of a vinyl hose was inserted into the styrofoam box and the other end passed through the head and led to an opening in the mouth from where sublimated CO_2 escaped. A vinyl glove was used to spread a thin layer of Tanglefoot insect trapping adhesive (gel type)[3] over the lining of the nostrils, the surface of the muzzle and the lips of the model. This material is a weather proof, odorless insect trapping adhesive which remains sticky in all kinds of weather.

The head of the model was returned to the laboratory after a days' exposure, and small globs of adhesive were scrapped from specific areas with a forceps. Individual samples stuck to the forceps were either examined directly with a dissecting microscope to identify the trapped insect fauna, or they were spread onto a piece of clear polyethylene before examination.

Results

Seven females were electrostimulated while viewed with a dissecting microscope. Most females only responded to electrostimulation once or twice, and they usually did so by forcefully spraying a group of larvae a distance of 5-20 cm. However, for 3 of 12 larvipositions observed, a female simply extruded the larvipositor and expelled, respectively, 48,50 and 90 larvae in a single large drop of clear uterine fluid. These large drops were watery (in contrast to the sticky drops expelled by *C. apicata* (Cogley and Anderson, 1981)), and almost immediately after expulsion, they began to trickle down the larvipositor and disperse into the pile (=hairs) around the base of the larvipositor. It was from these large drops that small groups of larvae were picked up with a camelhair brush.

Most larvae were forcefully expelled in a spray of about 20 tiny droplets, with each droplet containing from two to eight larvae. Most of the sprayed droplets landed on the microscope stage 5-10 cm in front of the electrostimulated females. Many of the upwardly sprayed droplets also hit the objective (7-10 cm above the females), and some hit other parts of the microscope. A few droplets landed on the laboratory bench up to 20 cm from an electrostimulated female. Because of the way an electrostimulated female usually was positioned at the front portion of the microscops stage, the ventral surface of the objective was situated much like the hypothesized target area on the ventral surface of the upper lip of the reindeer host would be exposed (see below).

The uterine fluid in which larvae were expelled was clear and of a watery consistency. Contrary to the more viscous sticky larval packet expelled by *C. apicata* (Cogley and Anderson, 1981), the uterine fluid of *C. trompe* did not stick to the pile on females nor to an insect pin or to brush bristles. When a pin was inserted into a newly-expelled drop of larvae the larvae simply slid off in the watery fluid as the pin was raised. If a pin were held horizontally while slowly removed from a drop of larvae, some larvae crawled along the moist pin until its surface dried and they then desiccated.

When larvae were picked up from uterine fluid on the bristles of a camelhair brush which was then oriented vertically at various angles they vigorously crawled downward; when the brush was oriented laterally the larvae rapidly crawled away from a source of bright light. Larvae behaved in this manner whether they crawled as individuals or whether several larvae in a uniform drop simultaneously crawled in the

[3]The Tanglefoot Co., Grand Rapids, MI., U.S.A. 49504.

same direction. Individually isolated larvae crawled at a speed of 4-5 mm/sec. When several larvae were picked up from the dispersed film of uterine fluid with a camelhair brush the moisture surrounding each larva often united and coalesced to form a small drop containing several wriggling larvae. When the brush was then held at even a slight angle the movement of the larvae within the drop resulted in the drop of larvae quickly "rolling" down a bristle.

Larvae individually separated on brush bristles became immobile and dried out in just a few seconds. However, after the larvae had been in a desiccated state for 1-2 minutes some revived when the bristles were immersed in a drop of physiological saline. On one occasion, 10 of 30 "desiccated" larvae began to move and quest after a few seconds in the saline. The others appeared dead. In another case, 30 of 48 such dried out larvae began to make questing movements after a few seconds in a drop of saline. A few larvae from several other groups that had momentarily dried out and stopped crawling, also revived after being put into a drop of physiological saline or human saliva. No larvae revived after 5 minutes of desiccation.

When groups of larvae obtained from dissected females were pipetted onto the muzzle of restrained reindeer in a single drop of physiological saline their wriggling and crawling resulted in the drop quickly spreading and drying. Such drops usually dried in just a few seconds as the fluid spread out on the thickly-matted hairs covering the surface of the muzzle. The smallest drops of 2-10 larvae dried in 2-5 sec., whereas the lager drops might remain moist for about 30 sec. Thus larvae were able to crawl only a few mm before becoming immobile and desiccating on the hairs of the reindeer's muzzle. Compared to their rapid locomotion along the bristles of a brush or the solid surface of a moist pin, the larvae appeared to experience more difficulty in crawling over the surface of the fine, soft hairs covering the muzzle. Perhaps it was more difficult for them to use their mouthhooks and ventral spines on a surface composed of numerous fine, loose hairs.

On several occasions captured females spontaneously larviposited in the ventilated holding vials. When this occurred the larvae continued to crawl around the interior of the vial for as long as the inside surface of the vial remained moist (as long as an hour in the maximum instance). When placed into drops of physiological saline in depression slides the larvae remained alive and active for up to 30 minutes, the longest time they remained in saline before used in experiments.

When drops of about 25 larvae were placed several cm into the nostrils (beyond the fringe of long guard hairs surrounding the entrance) the larvae were able to crawl laterally along the nostril passage. These larvae crawled more effectively along the surface of the slightly wrinkled, leather-like (hairless) lining of the inner nostril than larvae observed on the soft hairs of the muzzle. On two occasions a few larvae were seen crawling deep into the nasal passage until they disappeared from view. On these occcasions the reindeer responded with a few sneezes and snorts, and by closing and opening her nostril several times in rapid succession. These reactions appeared to be a response to irritation, probably caused by the movements of the large mouthhooks and ventral body spines used by larvae in locomotion.

When drops of larvae were pipetted onto the upper or lower lip of a reindeer the larvae quickly crawled out-of-sight into the mouth. The reindeer often responded to these larvae by licking the lips with its tongue and by rapidly moving and rubbing the upper and lower lips together. It also responded with some sneezes, snorts and head shakes. All larvae placed on the lips appeared to successfully enter the mouth.

Like deer models proviously used (Anderson, 1989), the reindeer model baited with CO_2 proved successful in attracting and inducing wild *C. trompe* females to larviposit. In two one-half day exposures of the model in 1987, we re-

covered 59 first-instar larvae. Only 4 of these were stuck a few cm outside the target area illustrated in Fig. 3. The CO_2-baited reindeer model thus proved successful in inducing several larviposition attackt by wild *C. trompe* females. All natural larvipositions onto the CO_2-baited reindeer model by wild females revealed that the *C. trompe* larvae were stuck only on the ventral surface of the protruding dorsal lip, usually in a small area below the philtrum (Figs. 2,3). Larvae trapped in the adhesive were compared to first instar larvae dissected from captured females to confirm their identification as *C. trompe*.

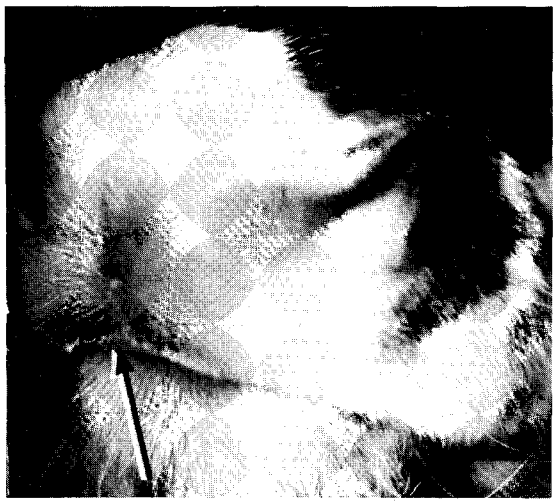

Figure 2. Close up view of the muzzle of a reindeer illustrating the exposed ventral surface of the protruding upper lip where all larvae expelled by wild female *C. trompe* were found. Arrow points to center of area where larvae were most commonly found stuck in the adhesive.

Finally, the CO_2-baited reindeer model, like the deer models used in California, appeared to also attract all parasitic Diptera that attack reindeer. Females of *Hypoderma tarandi* L. often landed and ovisposited on the hides covering the neck and body. On 10 August 1987, for example, we caught 6 *H. tarandi* females that were attracted to the model between 0900 and 1300 hrs. Members of all the blood-sucking

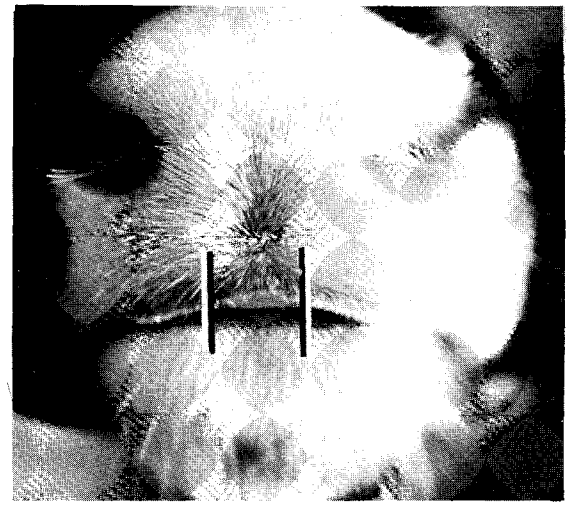

Figure 3. Same as figure 2. Almost all *C. trompe* larvae expelled by attacking females were found stuck within the target area enclosed by the two black lines.

fauna continually swarmed about, landed and crawled over most parts of the model. The numbers attracted were so large that the adhesive-coated areas soon became coverd with trapped black flies, mosquitoes and ceratopogonids (no-see-ums), whose sticky body surfaces had to be examined for *Cephenemyia* larvae after they were removed.

Discussion and conclusions

Our experimental studies, plus observations of attacking females by Espmark (1961, 1967), and by us, indicate that the natural method of host attack by *C. trompe* females involves a final behavior phase in which a female flies towards the mouth of a reindeer from the ground, or a hovering position below the nose. Because the upper lip of reindeer protrudes over the lower lip, the ground-launched attacks of *C. trompe* females seem effectively adapted for spraying larvae onto the ventral surface of the exposed upper lip of its reindeer/caribou host. Electrostimulation studies indicate that as a female approaches within about 5-15 cm of the mouth she forcefully expels a group of about 25-50 larvae in a spray comprised of about 10-20

watery droplets. The spray is directed at the ventro-central area of the upper lip, and the closer the female approaches a reindeer before expelling larvae, the tighter the spray pattern will be. When landing on the lips of the host the photonegative and thermopositive reactive larvae begin to immediately crawl into the mouth. Like the larvae of other *Cephenemyia* species (Cogley and Andreson, 1981), the *C. trompe* larvae probably crawl along the roof of the mouth and the tongue toward the posterior passages to the upper respiratory tract.

In the above respects, the manner of attack and expulsion of larvae by *C. trompe* are the same as that reported for *C. jellisoni* (Anderson, 1975; Cogley and Anderson, 1981), as is their crawling speed of 4-5 mm/sec. Two other features shared by these species that are associated with the expulsion of larvae in a spray of tiny droplets are, a watery uterine fluid and a large sclerotized utero-vaginal valve (Cogley and Anderson, unpubl. data). By contrast, *C. apicata*, which expels its larvae in a single packet, has a viscous, sticky uterine fluid and a small, narrow utero-vaginal valve (Cogley and Anderson, 1981; and unpubl. data). These and other anatomical modifications associated with viviparous reproduction and expulsion of larvae by nasopharyngeal bot flies of deer are being described by Cogley and Anderson (in prep.).

Although it has now been determined that *C. trompe* is the third *Cephenemyia* species whose larvae invade the host *per os*, one cannot completely exclude the possibility of the nostrils serving as an alternate portal of entry for expullsed *C. trompe* larvae. Because, on two occasions, drops of larvae experimentally placed deep within the nostrils were seen crawling out-of-sight deep into the nasal passage, it seems remotely possible that this could occur in nature. For example, if a reindeer happened to turn its head sideways at the moment a *C. trompe* female expelled its spray of larvae, it might be possible for some of the spray droplets to elude the guard hairs and enter an open nostril with enough velocity to land deep enough inside the nasal passage for the larvae to be able to crawl to the moist inner area before they would desiccate. Since larvae were not found in the nostrils of models, however, it seems unlikely that larvipositing females selectively home in on nostrils instead of the mouth.

With respect to the *per os* invasion of the host, there is a remote possibility that larvae in droplets that miss the lips and land on the muzzle might also be able to invade the host. If, for example, groups of 3-5 larvae in tiny droplets of sprayed uterine fluid landed on the furry surface of the muzzle within about 10 mm of the lips,it might be possible for some such larvae to crawl to the lips and into the mouth before they desiccated. However, because larvae in the smallest experimental drops of uterine fluid or physiological saline dried out and became immobile in just 2-3 sec., only larvae landing in a narrow zone around the lips might have such a chance to infect a host. Larvae experimentally dropped onto the muzzle did not crawl effectively on this furry surface, and none made it to the lips of the host.

Another remotely possible mode of infection is that the crawling activity of larvae might irritate the host enough to cause it to lick such larvae off the muzzle and into the mouth before they would desiccate, but we never saw this occur when drops of larvae were experimentally placed on the muzzle of a restrained reindeer. We therefore conclude that most reindeer become infected with *C. trompe per os* after first instar larvae have been sprayed onto the lips by a larvipositing female flying towards the muzzle from a ventral position.

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