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## EFFECTS OF *CEPHENEMYIA* SPP. (DIPTERA: OESTRIDAE) ON THE NASOPHARYNX OF BLACK-TAILED DEER (*ODOCOILEUS HEMIONUS COLUMBIANUS*)

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**ABSTRACT:** A study was conducted to determine gross and microscopic tissue changes in the nasopharynx of black-tailed deer (*Odocoileus hemionus columbianus*) infected with nasal bot fly larvae (*Cephenemyia* spp.). Paired retropharyngeal recesses were the preferred sites for the growing second and third stage larvae of two species of *Cephenemyia* (*C. apicata* and *C. jellisoni*). Retropharyngeal recesses distended into “pouches” that harbored up to 30 larvae. Pouches were oriented caudal-laterally toward the basisphenoid bone of the cranium. Lateral support of the pouch mass was provided by the stylohyoid bone. The laryngeal orifice was never occluded by the enlarged recesses. The distal pouch wall was relatively thin and remained uniform in thickness as expansion progressed. Occasionally, aberrant larvae were found protruding through the distal wall of the pouch. Disruption of the epithelium and submucosa by larval mouth hooks and integumentary spines were examined by scanning electron microscopy. Histological examination of infected recesses revealed substantial loss of epithelium and mucous glands. Enlargement of recesses into pouches resulted from fibrosis. Healing occurred after larvae egressed from the pouches. Degenerating mucous glands, epithelial metaplasia, epithelial desquamation, and intense inflammation were found near larvae. An eosinophilic exudate with a mixture of macrophages and erythrocytes was present in the lumen of the pouch. The presence of larvae within the pouch inhibited secondary bacterial infection and suppuration. Infection by larvae caused severe local trauma and intense tissue response.

**Key words:** Black-tailed deer, *Cephenemyia apicata*, *Cephenemyia jellisoni*, nasopharynx, nasopharyngeal bot fly, myiasis, *Odocoileus hemionus columbianus*, pathology, scanning electron microscopy.

### INTRODUCTION

Nasopharyngeal myiasis of deer (*Odocoileus* spp.), elk (*Cervus canadensis*), moose (*Alces alces*), and reindeer (*Rangifer tarandus*) is predominantly the result of infection with the larvae of nasal bot flies of the genus *Cephenemyia*. Prevalence of the infection in herds of deer and elk may reach 75% (Capelle and Senger, 1959) to 95% (Sugar, 1974). The eight species of nasopharyngeal bots are obligate parasites as larvae. They gain entry to the host through the efforts of the swift flying adults that larviposit directly onto the artiodactyl. Larvae of two species of *Cephenemyia* are known to enter the deer through the mouth after being placed or sprayed onto the nose (Cogley and Anderson, 1981). All three larval stages of *Cephenemyia* spp. live on the mucosal sur-

face of the host's respiratory tract. The trachea, lungs, or sinuses of the host are specific sites of infection for the first stage larvae. Growth of second and third stage larvae proceeds within two retropharyngeal recesses that enlarge into large gall-like pouches from minute depressions of the pharyngeal wall.

The importance of infections with the larvae of *Cephenemyia* spp. to the well-being of deer is not understood fully. It is accepted widely that adults of *Cephenemyia* spp. cause gadding of deer or elk, whereas migrating third stage larvae produce trauma within upper respiratory tract tissue. Weight loss (Nordkvist, 1967; Barth et al., 1976) and death (Walker, 1929; Cowan, 1946) of the host has been attributed directly to the bots found in the pharyngeal recesses. Larvae of *Cephenemyia* spp. have been implicated in the devel-

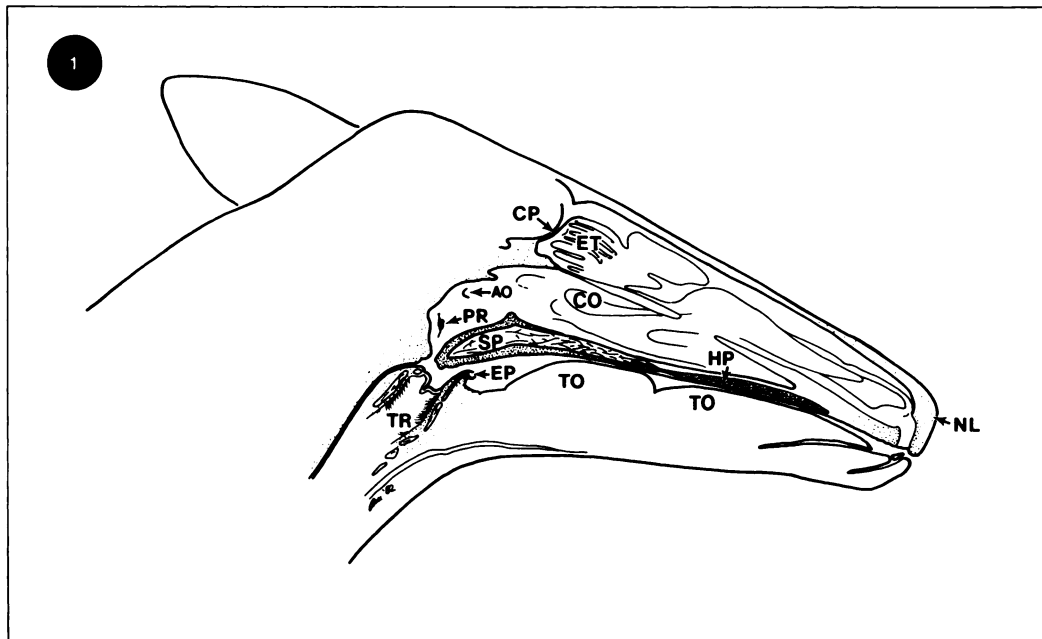


FIGURE 1. Midsagittal section of the head of a black-tailed deer showing the relationship of the retropharyngeal recess to the oral cavity and upper respiratory tract. AO, auditory tube opening; CO, conchae; CP, cribiform plate; EP, epiglottis; HP, hard palate; NL, nasal labium; PR, retropharyngeal recess; TO, tongue; and TR, trachea.



FIGURE 2. The opening of a retropharyngeal recess in a black-tailed deer is demonstrated (arrow) after more than 30 third stage larvae (L) of *Cephenemyia* spp. were removed. Tongue of deer (TO).

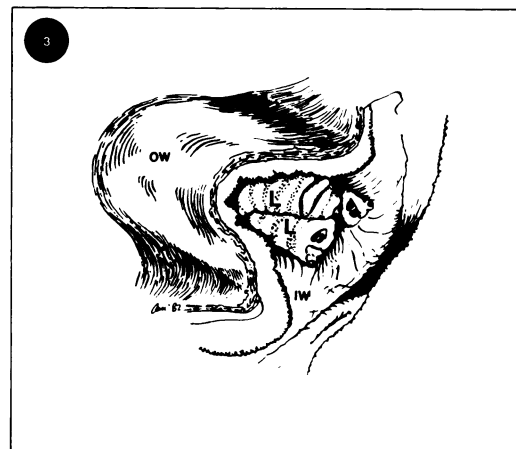


FIGURE 3. Tightly packed third stage larvae of *Cephenemyia* spp. within an enlarged retropharyngeal recess of a black-tailed deer. The cutaway view illustrates both the outer walls (OW) on the visceral side of the recess, and the mucosal surface wall (IW) exposed to the nasopharynx. The anus and posterior spiracles of the *Cephenemyia* larvae (L) are all that can be seen at the opening of the recess.

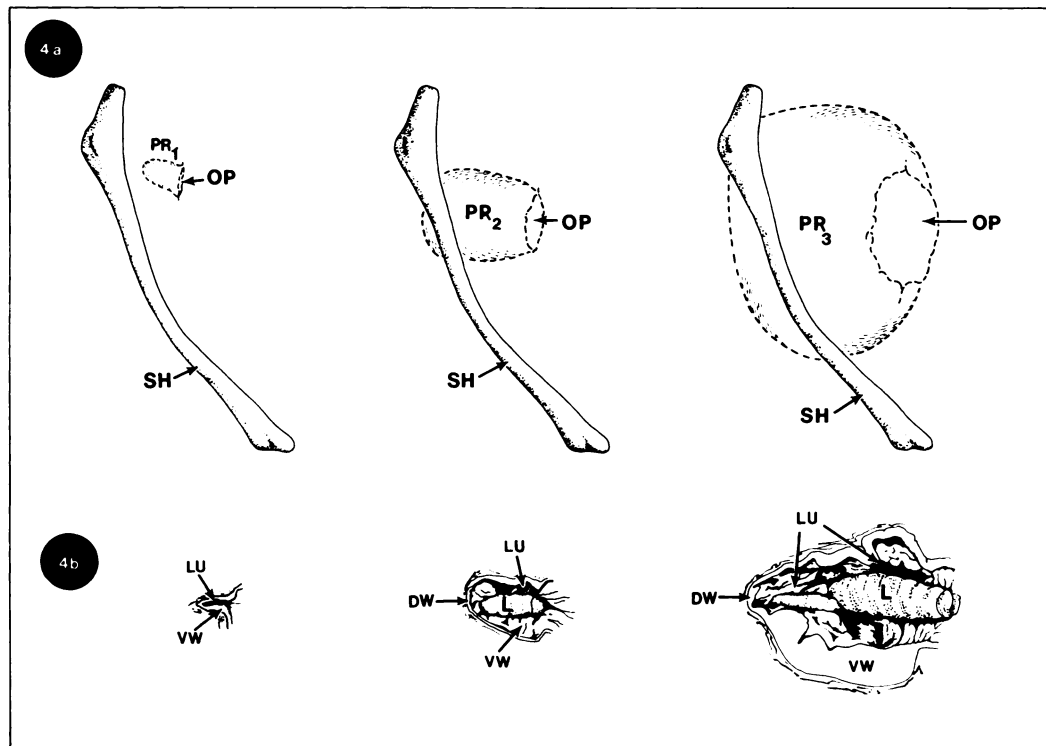


FIGURE 4. Differences between normal retropharyngeal recesses of black-tailed deer and those infected with *Cephenemyia* larvae are shown in comparison to the stylohyoid bone (drawn to scale). 4a. The undisturbed retropharyngeal recess (PR<sub>1</sub>) has a smaller opening (OP) and overall size compared to infected retropharyngeal recesses (PR<sub>2</sub>, PR<sub>3</sub>). The growth of 22 larvae in retropharyngeal recess 3 has formed an enlarged pouch with a nearly circular opening (OP). 4b. Cutaway view of PR<sub>1</sub>, PR<sub>2</sub>, and PR<sub>3</sub> of black-tailed deer. In PR<sub>2</sub> and PR<sub>3</sub> note the relative thinness of the distal wall (DW) as compared to the thick ventral wall (VW). Larvae (L) of *Cephenemyia* spp. in the lumen (LU) of PR<sub>2</sub> and PR<sub>3</sub> are oriented along the long axis.

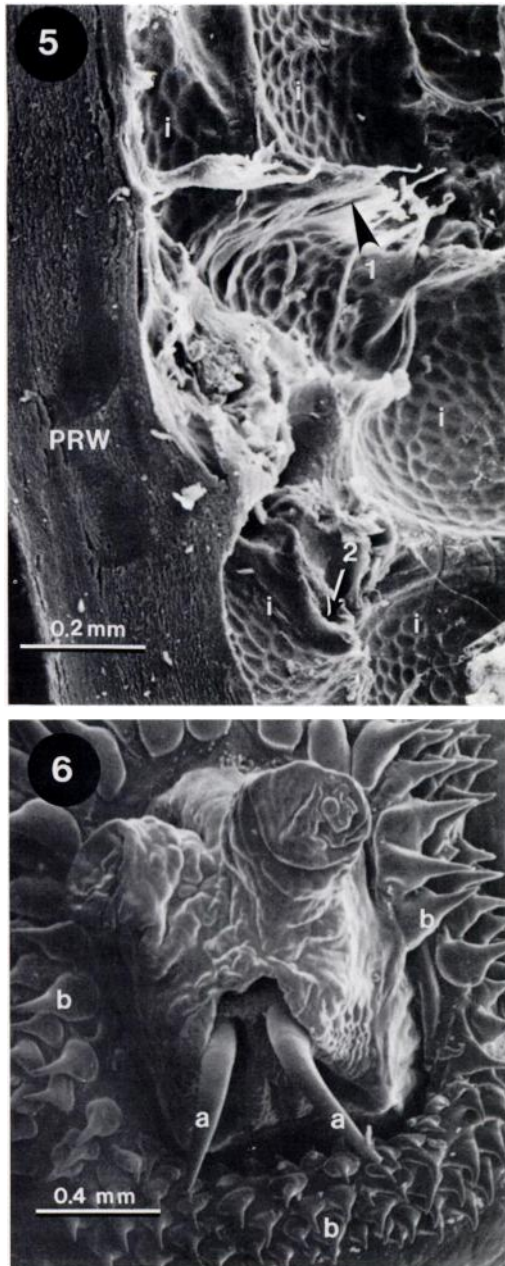
opment of cerebral abscesses (Johnson et al., 1983) and meningitis. Other studies indicate that there are no ill effects in deer harboring large numbers of larvae of *Cephenemyia* spp. (Bennett, 1962; Hair et al., 1969; Samuel and Trainer, 1971). Although many reports draw attention to the larvae within the retropharyngeal recesses, little is known about the pathology that results from such infections. This paper describes the pathologic changes induced by larvae of *Cephenemyia* spp. within the retropharyngeal recesses of black-tailed deer (*Odocoileus hemionus columbianus*).

#### MATERIALS AND METHODS

Tissue samples were obtained from 28 black-tailed deer within 0.5 hr of death from the man-

aged deer population at the University of California Field Station, Hopland, California (38°58'N, 123°08'W). Specimens were obtained also from 10 unthrifty deer living in overcrowded conditions on Angel Island, California (37°52'N, 122°25'W). The oral cavity of each deer was opened and the nasopharynx, soft palate, and caudal aspect of the tongue were removed. The tissue was immediately placed in buffered 10% formalin at 4 C. Cold fixation was used to prevent larvae from leaving the pharyngeal recesses. Presence or absence of larvae, dimensions of the recess, and the position of the recesses were noted. Calibrated syringes were used to determine the size of recesses by recording the volume of fluid needed to fill their cavities.

After the recesses and surrounding tissues were fixed, they were cut in half along their long axis. One-half was prepared for scanning electron microscopy (SEM) and the other for histological examination. The tissues for SEM were dehy-



FIGURES 5, 6. 5. Scanning electron micrograph of the retropharyngeal recess wall (PRW) of black-tailed deer. The body impression (i) of a *Cephemyia* spp. larvae is retained by crushed cilia. Undisturbed cilia remain in their normal upright position (arrow 2). 6. Scanning electron micrograph of the cephaloskeleton of a third stage *Cephemyia jellisoni* larva. The mouth hooks (a) and body spines (b) cause severe damage to host tissue.

drated through 30, 50, 70 and 95% ethyl alcohol and placed in absolute alcohol. Tissues were then critical point dried, mounted on studs with Pelco® (Ted Pella Company, Tustin, California 92680, USA) colloidal silver, coated with gold palladium, and examined with SEM. Tissues for light microscopy were prepared similarly, except after dehydration the tissue was embedded in paraffin, sectioned at 6  $\mu$ m, and stained with hematoxylin and eosin or with Mowry's alcian blue-PAS procedure for mucopolysaccharides (McManus and Mowry, 1960).

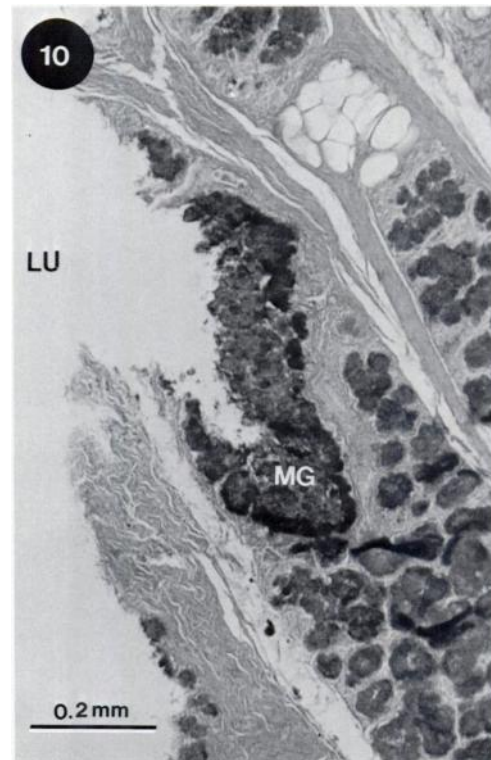
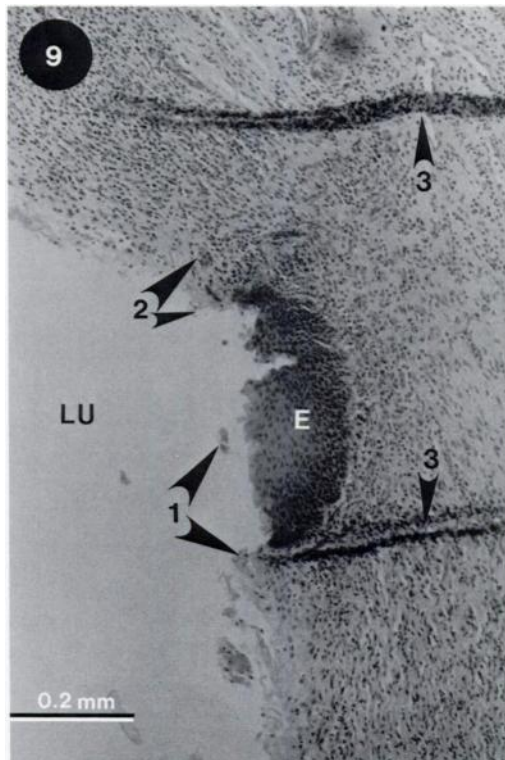
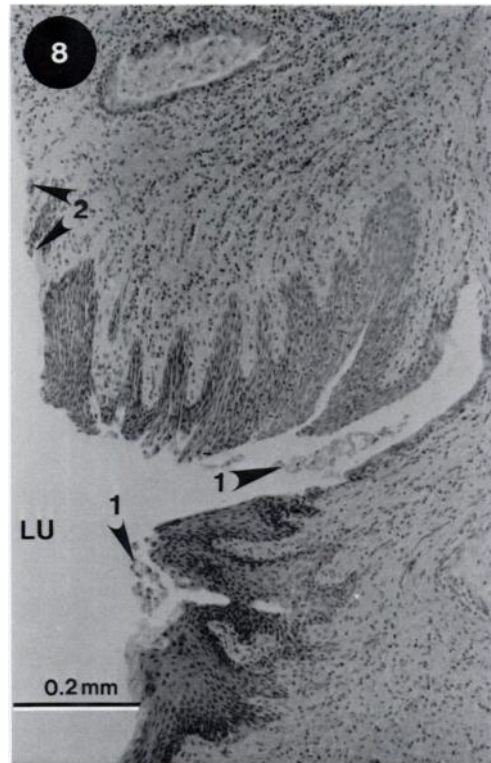
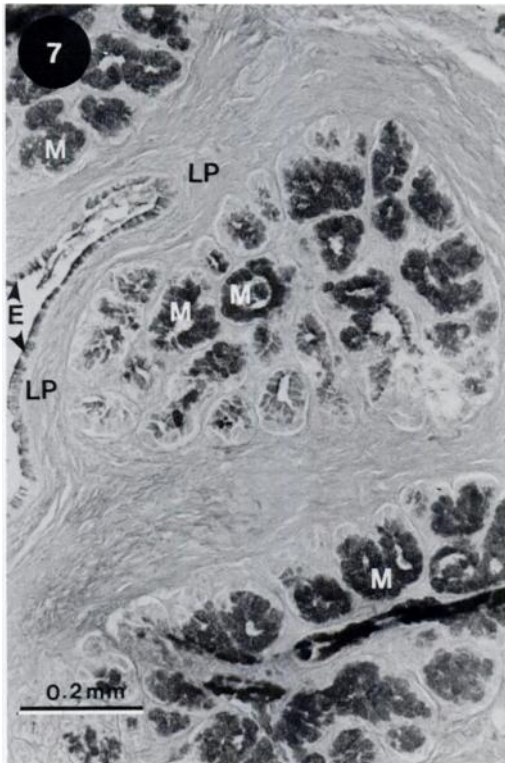
## RESULTS

Retropharyngeal recesses that were not infected with larvae of *Cephemyia* spp. appeared as small elliptic slits (Fig. 1) on each side of the nasopharynx. Observation of the retropharyngeal recesses in living deer was not possible because of their position between the soft palate and auditory tube opening. Undisturbed recesses had a volume averaging  $0.02 \pm 0.01$  ml. Enlarged pouches extended rostrally to the base of the cranium near the basisphenoid bone.

The opening to a recess infected with larvae of *Cephemyia* spp. was widened and nearly circular. As many as 30 larvae, a mixture of both *C. apicata* and *C. jellisoni*, were found within the recess (Fig. 2). Larvae did not protrude into the pharynx. They were tightly packed into the lumen of the retropharyngeal recess, resulting in limited movement and producing a turgid "pouch." Larvae were capable of rapidly leaving pouches that had been placed in formalin. Cold fixative prevented such movement. Heads of larvae were buried deeply within the pouch, while the posterior spiracles of larger larvae were visible at the recess opening (Fig. 3).

Greatly enlarged pouches swelled caudally and laterally away from the larynx and airway up to the stylohyoid bone (Fig. 4a). The stylohyoid supported the bulky mass of the pouch and restricted its further lateral enlargement. An infected recess could become cavernous, with its opening extended downward to the free border of the soft palate and contiguous with the





recess of the opposite side. Although expansion of the recess into a pouch was considerable, the distal wall of the recess remained a uniform 1.0–2.0 mm in thickness (Fig. 4b). The outer wall of the pouch (visible on the visceral side of the recess) was smooth. Its appearance was much the same as the cattle warble (*Hypoderma bovis*) viewed from the subcutaneous side of the hide. The inner surface of the recess was roughened, convoluted and contained plaques of tissue crosshatched with grooves and mottled with pits. On two occasions, the bodies of third stage larvae were found partially extending through the distal wall of the recess. The damage to tissue of the distal wall surrounding the aberrant larvae was healed. The volume of enlarged recesses ranged from 4.0 to 10.0 ml ( $\bar{x} = 7.0 \pm 2.5$ ).

Scanning electron microscopy of infected pouches revealed that the epithelial plaques visible to the naked eye were thickened, elevated, and devoid of cilia. Deep fissures extended throughout the bed of cilia and into the submucosa. Pouches with little tissue disruption visible to the naked eye had long cilia. Cilia were compressed into impressions of the larval body wall (Fig. 5), and were eroded focally where mouth hooks were embedded. Recurved body spines and mouth hooks (Fig. 6) prevented easy removal of the larvae from the pouch. Nevertheless, the spines and mouth hooks were only loosely attached to the mucosa of the pouch.

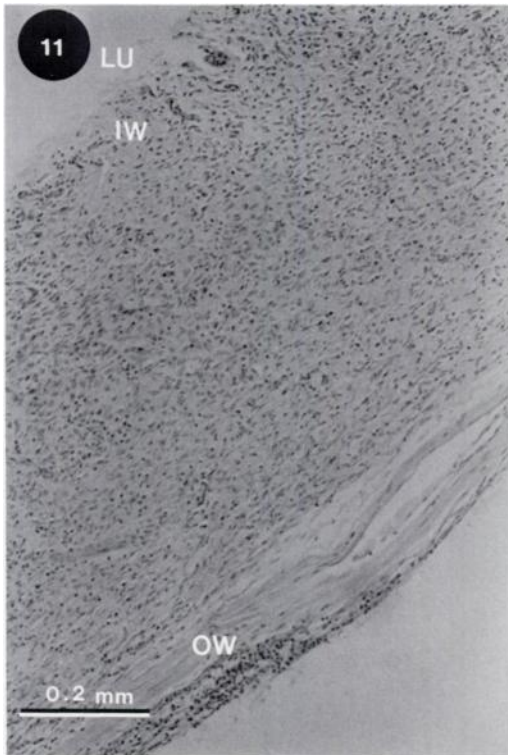
Histological examination of uninfected recesses revealed epithelial tissue lining their entire inside wall. The predominant

tissue was ciliated pseudostratified columnar epithelium. The lamina propria and submucosa consisted of loose connective tissue infiltrated with lymphocytes (Fig. 7). Alcian blue-PAS stain revealed that the simple branched tubular acinar glands in the submucosa were filled with mucopolysaccharide.

The surface of infected recesses had extensive loss of epithelium (Fig. 8). The edges of epithelia adjacent to large denuded areas often showed active desquamation ultimately leaving only small epithelial patches of pseudostratified squamous epithelium (Fig. 9). Many larvae *in situ* rested well below the level of epithelia, with their body spines embedded in the lamina propria. Occasionally erosion of the recess wall extended to or below the level of the mucous glands in the submucosa (Fig. 10). Throughout the wall of the recess, those mucous glands not in direct contact with larvae were degenerating. Glands near the entrance of the recess appeared unaffected. In large pockets mucous glands were rarely encountered and the wall was composed of slightly vascular fibroblastic tissue (Fig. 11). Vascularization was more extensive in the thicker ventral wall of the recess than in the dorsal or distal walls (Figs. 11, 12). A diffuse inflammatory response of eosinophils, plasma cells, and lymphocytes was present in the walls of enlarged pockets (Fig. 12). Eosinophils and erythrocytes were found within the exudate along the inner lining of the recess. Neutrophils were rare in the exudate and accounted for the notable absence of gross suppuration.

←

FIGURES 7–10. 7. Normal appearance of the retropharyngeal recess wall of black-tailed deer. The mucosa consists of a layer of epithelia (E) underlined by lamina propria (LP). The mucous glands (M) are situated in the submucosa. H&E. 8. Exudate (arrow 1) and epithelial erosion (arrow 2) in a recess of a black-tailed deer infected with *Cephenemyia* spp. larvae. LU = lumen of recess. H&E. 9. Desquamation of epithelia in the recess of a black-tailed deer (arrow 1) has left small patches of epithelia (E) within a denuded, inflamed mucosa (arrow 2). Note folding of tissue which occurs naturally in the emptied recess (arrow 3). LU = lumen of recess. H&E. 10. Destruction of mucous glands (MG) and the surrounding lamina propria by *Cephenemyia* spp. larval activity in the retropharyngeal recess lumen (LU) of black-tailed deer. Alcian blue-PAS stain.



FIGURES 11, 12. 11. Distal wall of an infected retropharyngeal recess of a black-tailed deer. The homogeneous appearance is due to the lack of epithelia in the inner wall (IW) and lack of vascularization in the outer wall (OW). LU = lumen of recess. H&E. 12. Ventral wall of heavily infected and greatly enlarged recess of a black-tailed deer. The inner wall (IW) of the recess is denuded and densely infiltrated by lymphocytes (arrow 1). Vascularization is pronounced near the outer wall (OW). Folding of the recess wall is present (arrow 2). LU = lumen of recess. H&E.

Enlarged recesses shrank rapidly as larvae exited or were removed. The shrinking tendency of the tissue was depicted in histological sections where folds in the recess wall occurred in a regular fashion (Figs. 9, 12).

On several occasions superfluous tissue was found posterior to normal appearing recesses. This tissue contained remnants of mucous glands within collapsed, folded recess walls.

Infected deer from Angel Island and Hopland had similar pathologic changes in their recesses.



## DISCUSSION

Soon after first stage larvae of *C. apicata* and *C. jellisoni* enter the mouth of deer, the second stage develops in the sinuses, or lungs. Second stage larvae then make their way into the lumen of the retropharyngeal recesses. Here, both species coexist as they develop to third stage larvae. The volume of the lumen in its normal state will accommodate only two or three early second stage larvae.

The mouth hooks and body spines of larvae are important in disrupting and causing enlargement of the recesses. As growth of larvae proceeds through the third stage, the lumen of the retropharyngeal recess typically enlarges into a pouch with a volume of 4–10 ml. This amounts to as much as a 500-fold increase in the volume of the recess. Bennett (1962) reported a pouch in a white-tailed deer (*Odocoileus virginianus*) that had reached a volume of 75 ml. During pouch enlargement, substantial changes occur within the pouch, including (1) varied degrees of excoriation and damage to the lining epithelium and lamina propria; (2) inflammation of the mucosa and submucosa; (3) mucous gland degeneration; and (4) extensive elongation of recess walls through fibrosis.

The host response of fibrosis is preceded by epithelial metaplasia. In greatly disturbed areas, ciliated pseudostratified columnar epithelial cells are replaced by stratified squamous epithelium. Damage by larvae of *Cephenemyia* spp. is confined primarily to the mucosal surface, but in severe infections it extends to the upper portions of the submucosa and mucosal glands.

Aberrant larvae may harm the host when they penetrate the enlarged retropharyngeal pouches near the cranium. The thin distal wall of the recess seems to be a susceptible area of penetration. Other investigators have noted aberrant larvae of *Cephenemyia* spp. (Cameron, 1932; Dudzinski, 1970) or, larvae that have been

aspirated into lungs after emerging from the recesses (Nordkvist, 1967).

It has been postulated that healing of the retropharyngeal pouches occurs in 2–3 wk after the larvae leave the recesses (Bennett, 1962). Evidence of rapid healing is supported by the appearance of a morphologically normal recess before complete reorganization of the old collapsed pouch has been accomplished. The considerable damage caused by larvae in the retropharyngeal pouch is offset by the rapid regeneration in affected epithelial tissues.

The degeneration of mucous glands in either direct or indirect contact with growing larvae reduces the likelihood that these glands function as a source of larval nutrition in the pouch. Degeneration of glands may be due to larvae exuding toxic material into the recess. Alternatively, pressure exerted by the growing larvae on the recess walls could cause necrosis of the glands.

Lack of suppuration in the retropharyngeal recesses infected with larvae of *Cephenemyia* spp. has been noted by others (Bennett, 1962; Dudzinski, 1970). It would be expected that defecation within the cavity by smaller second stage larvae, and exuviae produced during the second to third stage molt would promote suppuration. Lack of suppuration in the warble of the cattle bot, *Hypoderma bovis*, and the rabbit bot, *Oestroychia leporina* (Hypodermatidae) has been found also (Wolfe, 1959; Rietshel, 1979). In infections with the rodent bot fly, *Cuterebra angustifrons*, Payne and Cosgrove, 1966 found that "bacterial growth is rapid (in the cavity) after the bot leaves." Bacterial growth in the recess is in some way suppressed by larvae of *Cephenemyia* spp. "Surgical maggots" used in the 1930's to disinfect wounds (Robinson and Norwood, 1933) inhibit bacterial growth in three ways: (1) larval ingestion and subsequent digestion of bacteria; (2) ingestion of necrotic tissue that could be used as bacterial substrate;

and/or (3) stimulation of host tissue secretion that washes away bacteria. The latter was attributed to the effects of larval waste products, such as urea and allantoin (Robinson, 1937) or ammonia and calcium carbonate (Stewart, 1934).

It is evident from this study that severe pathologic changes in the retropharyngeal recesses of black-tailed deer occur as a result of infection with larvae of *Cephenemyia* spp. Discomfort to deer is probably due to the inflammatory reaction within the enlarged recess. Severe disease or death is unlikely because of (1) the lack of supuration in the recess; (2) the lack of involvement of surrounding tissues; (3) rapid healing of the retropharyngeal recess; and (4) the ability of the nasopharynx to accommodate greatly expanded retropharyngeal recesses.

Perhaps under extremely stressful conditions, beyond those imposed on the Angel Island deer, *Cephenemyia* spp. may cause great harm to their hosts. Such conditions exist when the local healing abilities of the host are retarded. The healing abilities of the host are seen from this study to be vital in the production of an adequate pouch, and in reducing the superfluous tissue of the pouch once larvae have exited. Such conditions may have been encountered where debilitation and ultimate death of the deer infected with *Cephenemyia* spp. were reported (Fitch, 1928; Cowan, 1946; Cheatum, 1951).

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