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## ***Onchocerca cervicalis* in horses: dermal histopathology**

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### **Summary**

A histopathological study of ventral midline skin from midwestern U.S. horses with and without onchocerciasis due to *Onchocerca cervicalis* found perivascular mononuclear dermatitis as the most consistent difference between the two groups. Seasonal variation in parasite numbers or cellular influxes was not observed. Eosinophilic dermatitis was observed in horses with onchocerciasis and dermatitides of unknown etiology.

**Key words:** onchocerciasis; equine; pathology; *Onchocerca cervicalis*; perivascular mononuclear dermatitis.

### **Introduction**

Onchocerciasis in horses occurs with a cosmopolitan distribution (Engelkirk et al., 1982; Rabalais et al., 1974). Dermatitis in horses caused by *Onchocerca cervicalis* is characterized clinically by pruritis, scaling, and excoriations of the ventral midline, chest, withers, neck, and face. The most common site of *O. cervicalis* microfilariae (mf) in the horse is the dermis of the ventral midline (Mellor, 1973a; Rabalais and Votava, 1974).

Histopathological diagnosis of this disease is confirmed by the presence of mf in skin biopsies. *O. cervicalis* mf frequently tend to “clump” or “nest” together in the upper dermis adjacent to sweat glands or hair follicles (Mellor, 1973a; Stannard and Cello, 1975). Descriptions of microscopic skin changes in horses have been minimal, because most surveys have been limited to determining the prevalence of the infection.

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In a study of English horses, Mellor (1973b) observed a mild perivascular infiltration of round cells and eosinophils, neither of which was concentrated around the mf; these microscopic changes could not be attributed to the presence of the parasite. Similar histopathologic findings have been briefly described for *O. cervicalis* infected horses in North America. Perivascular cuffing of lymphocytes, plasma cells, and, occasionally, eosinophils were the only findings that differentiated mf positive and mf negative skin samples from western U.S. horses (Stannard and Cello, 1975). A study of mf positive Canadian horses with clinical dermatitis revealed moderate acanthosis, with mononuclear and eosinophilic dermatitis (Marcoux et al., 1977).

Seasonal variation in clinical signs has been recognized in Texas and English horses but not in California horses (Mellor, 1973a; McMullan, 1972, 1977; Stannard, 1972). In English horses, mf were found in the deeper levels of the dermis (1–2 mm from the epidermis) in the winter (Mellor, 1973a). In Texas horses, lower microfilarial counts were observed in skin biopsies obtained in the summer than in the winter (McMullan, 1972, 1977).

The aims of this study are: 1. to compare the histopathologic features of ventral midline cutaneous biopsies obtained during the winter and summer from horses with and without *O. cervicalis* mf, and 2. to determine if unique histopathologic features could be observed in skin biopsies obtained in the summer from mf positive horses with clinical histories and signs of pruritic dermatitis versus those obtained from mf negative horses.

## Materials and Methods

Onchocerciasis was diagnosed in midwestern U.S. horses either by necropsy examination of the nuchal ligament (Schmidt et al., 1982a), after incubation of abdominal skin samples overnight in saline (Camp and Leid, 1983), or by the histopathological examination of abdominal skin biopsies (Schmidt et al., 1982b). In most cases, at least 2 of these 3 methods were used. *O. cervicalis* was identified by the criteria of Bain (1975) and Engelkirk et al. (1982).

Skin samples from 30 horses with onchocerciasis and 19 horses without onchocerciasis were divided into the following groups for study: Group A – 20 animals, no clinical signs of dermatitis; ventral midline skin sampled in the early summer (May, June and July); Group B – 16 horses treated the same as group A except that these animals were biopsied in the winter (December, January and February); Group C – 13 horses with pruritic dermatitic lesions biopsied in the early summer. Horses from all groups were further categorized with respect to the presence (+) or absence (-) of *O. cervicalis* mf. The majority of biopsies from the 49 horses used in this study were repeat samples. The presence or absence of the infection was confirmed at necropsy by examination of the nuchal ligament (Schmidt et al., 1982a); however, not all nuchal ligaments from Group C horses were available for examination.

Skin biopsy samples were fixed in 10% buffered formalin and processed for light microscopy (Schmidt et al., 1982a, b). Replicate tissue sections were stained with haematoxylin and eosin or Giemsa. Replicate sections from Group C were also stained with hematoxylin-phloxine-metanil-yellow (HPMY) (Gipson and Kimball, 1979) as a means of detecting fibrinoid deposits, a histopathologic indication of immune complex deposition.

The presence and location of mf in the dermis, and the type and location of inflammatory cells and dermal structural abnormalities were noted for each section.

## Results

A summary of our findings is shown in Table 1. There was no difference in the histopathologic reactions observed in the skin of *O. cervicalis* positive horses from Group A and Group B, whether the biopsy was obtained in the early summer or in the winter. Mf were most consistently located adjacent to the base of the hair follicles and sweat glands, regardless of the season (93% for these groups). These findings are similar to those of Stannard and Cello (1975), but are in contrast to those of Mellor (1973a).

Fibrinoid material suggestive of immune complex deposition was not identified from HPMY stained tissue sections in any horse from Group C.

Perivascular mononuclear (lymphoid cells, macrophages) dermatitis (Fig. 1) was present in 90% (27/30) of the horses with mf and in 5% (1/19) of the horses without mf.

Eosinophils, often associated with parasitism and allergic reactions, were present in only 50% (15/30) of the horses with mf (Fig. 2). Eosinophilic dermatitis (Fig. 3) was present in 100% of the horses with clinical dermatitis and mf but this particular cell type was also present in 33% (2/6) of the horses without mf and still presenting with clinical dermatitis (Table 1, Group C). Increased mast cell numbers were observed in only one horse with both dermatitis and mf (Fig. 4).

Table 1. Summary of histopathological findings from skin biopsies of horses with and without microfilariae (mf)

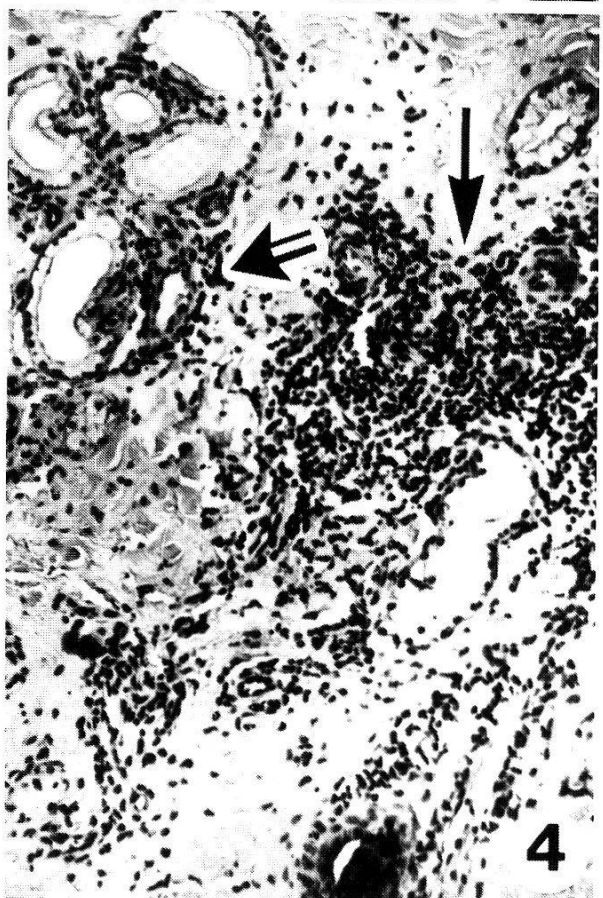
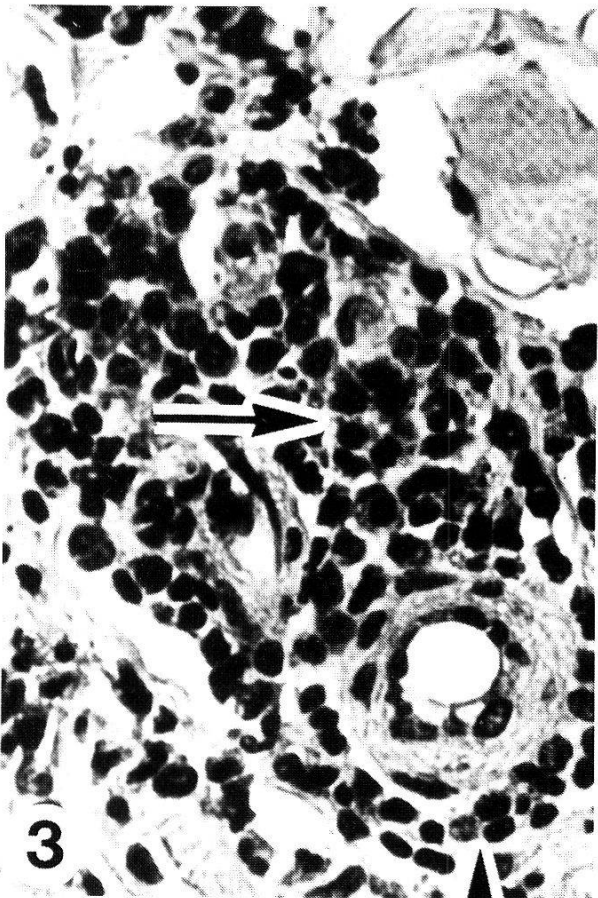
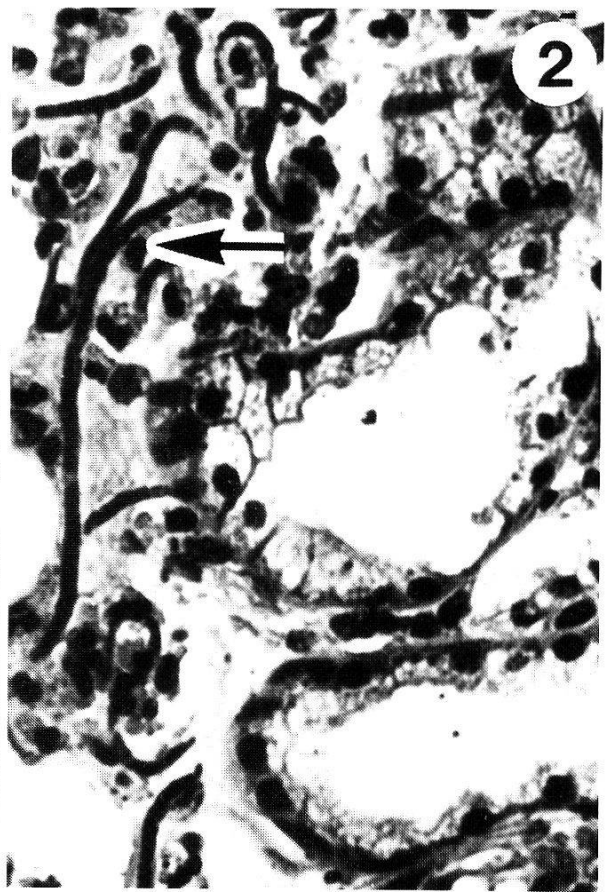
	<i>Onchocerca cervicalis</i> mf (+)					<i>Onchocerca cervicalis</i> mf (-)				
	Total no.	PMD <sup>1</sup>	ED <sup>2</sup>	DMD <sup>3</sup>	Normal	Total no.	PMD	ED	DMD	Normal
<i>Group A</i>										
Summer	13	10	4	0	3	7	0	0	2	5
<i>Group B</i>										
Winter	10	10	4	0	0	6	0	0	0	6
<i>Group C</i>										
Dermatitis, Summer	7	7	7	6	0	6	1	2	2	0
Total*	30	27	15	6	3	19	1	2	4	11

<sup>1</sup> PMD = perivascular mononuclear dermatitis (lymphoid cells, macrophages)

<sup>2</sup> ED = eosinophilic dermatitis

<sup>3</sup> DMD = diffuse mononuclear dermatitis

\* Totals may exceed the number of horses because more than one finding was present in the same horse.



## Discussion

Neither the season of the year nor the presence of insect vectors in the environment had an apparent effect on the histopathologic features of the equine skin studied (Table 1, Group A vs B). Biting midges of the genus *Culicoides* may be the vector for *O. cervicalis* (Mellor, 1974, 1975; Collins and Jones, 1978), although *Aedes aegypti* is an acceptable surrogate host for *O. cervicalis* and other *Onchocerca* species as well (Lok et al., 1980). In Michigan, however, neither of these vectors are present during the winter and transmission presumably does not occur. It is not known whether *Culicoides* sp. or *A. aegypti* are the insect vectors for *O. cervicalis* in the midwestern U.S.

*O. cervicalis* mf are not consistently associated with clinical signs of dermatitis in the horse. McMullan (1972, 1977) proposed that 1. the numbers of mf within the tissues are directly proportional to the severity of the disease and 2. only dead or dying mf are capable of inducing the disease state. Our findings in 3 horses with clinical dermatitis and mf lead us to conclude that these hypotheses may not be entirely correct. Two horses had clinical and histopathological dermatitis, but only 1–3 mf were observed per tissue section. Furthermore, the most severe dermatologic changes in human skin are associated with reduced numbers of mf (Henson et al., 1979). The second hypothesis might explain the presence of severe skin disease in cases with few dermal mf, because dead and dying mf would be in various stages of deterioration and, therefore, unrecognizable at the level of the light microscope. This hypothesis would not apply to one horse in Group C (Table 1), which possessed many viable mf and also had an intense and severe clinical and histopathological dermatitis.

Host inflammation does not regularly occur around the adult parasite in the nuchal ligament (Schmidt et al., 1982a), around ocular mf (Schmidt et al., 1982b), or around dermal mf (Table 1, Group A). We offer another hypothesis to explain the presence of mf disease variations in horses infected with *O. cervicalis* and human patients infected with *O. volvulus*. We propose that the parasites are actively suppressing the host immune response either locally or systemically.

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Fig. 1. Ventral midline dermal onchocerciasis from a horse without clinical dermatitis sampled in June (Group A+). Microfilariae are located near base of hair follicle (→) and a mononuclear perivascular dermatitis is present (⇒). The section is stained with H and E, ×150.

Fig. 2. Microfilariae surrounded by eosinophils in the sweat glands of the deeper dermis (→). The section is stained with H and E, ×400.

Fig. 3. A perivascular mononuclear and eosinophilic dermatitis are also evident (→). The section is stained with H and E, ×400.

Fig. 4. Dermal onchocerciasis from a horse with clinical dermatitis sampled in July and containing elevated levels of mast cells (Group C+). A diffuse (→) and perivascular (⇒) mononuclear dermatitis with few tissue microfilariae is present (H and E, ×150).

Modulation of the host's immune system has been suggested previously for larval cestodes (Leid, 1982; Leid and Williams, 1979). The immune system can, itself, modulate the generation and release of the chemical mediators of inflammation (Austen, 1979). These same chemical mediators are responsible for the development of both acute and chronic clinical lesions occurring in the dermatitides. The mechanisms by which mf might modulate host inflammation are currently not known. Because little host inflammation occurred immediately adjacent to the parasite in most skin biopsies, parasite-dependent modulation may be occurring in *O. cervicalis* infections. Whether this is correct remains to be seen, but this modulation would explain the inconsistent host reaction around the parasites.

Dermal perivascular mononuclear dermatitis was a consistent histopathologic feature in summer or winter biopsies from horses with dermal mf. In addition, eosinophils were variably present in the dermal lesions. The mechanism responsible for the infiltration of these eosinophils is unknown, although a variety of chemotactic factors have been suggested, including the generation of C5a/C5a des arg from the complement protein C5 (Camp and Leid, 1983). It is also possible that a parasite-lymphocyte interaction occurs, resulting in the production of lymphokines chemotactic for the host eosinophil. This has been shown in mice sensitized to *Schistosoma mansoni* antigens (Green and Colley, 1976; Lewis et al., 1977). Therefore, the interactions of the lymphocyte, macrophage, eosinophil and mast cell/basophil with the living or dying mf, regardless of the total numbers of mf present in the tissues, may determine whether clinical disease occurs in either *O. cervicalis* infections in the horse or *O. volvulus* infections in man.

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