PATHOLOGY OF THE MUSCLEWORM, PARELAPHOSTRONGYLUS ODOCOILEI (NEMATODA:METASTRONGYLOIDEA), IN MOOSE

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Abstract:

Parelaphostrongylus odocoilei is a muscleworm common in mule deer, Odocoileus hemionus hemionus, of western Alberta. Its effects in alternate hosts are essentially unknown. Two moose calves, Alces alces, were each given either 300 or 800 third-stage larvae of P. odocoilei. At necropsy particular attention was given to gross lesions and samples for histopathologic study. Gross lesions in moose consisted of myositis in the back and hind quarters accompanied by general softening of tissue, lymph node hypertrophy and petechial haemorrhage throughout the lungs. Histopathologic examination confirmed chronic progressive myositis in skeletal muscles and increased activity of the lymphoid system. Atelectasis, interstitial pneumonia and interlobular oedema were common in lung sections. Severity of infection in moose relative to that in mule deer is discussed briefly.

Differential host susceptibility or host specificity is a common feature of parasitic infections (Holmes 1976). In 1964, Anderson showed that moose (Alces alces) of North America are particularly susceptible to insult as a result of infection with meningeal worm, Parelaphostrongylus tenuis. Adult worms enter the central nervous system and cause extensive damage to nervous tissue. White-tailed deer (Odocoileus virginianus) can apparently harbour the worm with little effect (Anderson 1963).

Other members of the genus Parelaphostrongylus are also present within the normal range of moose yet their effect on moose is unknown. Recently, Platt and Samuel (1978) reported a muscleworm, P. odocoilei, in mule deer (O. hemionus hemionus) of western Alberta. Pybus and Samuel (in press) report P. andersoni, also a muscleworm, in white-tailed deer of southeastern British Columbia. Since moose share ranges with mule deer in western Alberta (pers. observ.) and white tails in southeastern B.C. (G. Tipper pers. comm.), the potential exists for exposure of moose to muscleworms. The following study was undertaken to determine pathological effects of P. odocoilei on moose calves. Susceptibility and severity of infection in moose and mule deer are compared briefly.

MATERIALS AND METHODS

In 1978, 2 moose approximately 4-6 weeks old were received from the Fish and Wildlife Division of the Alberta Department of Energy and Natural Resources. Animals were initially bottle-fed a 2:1 mixture of whole milk and evaporated milk for the first 2 weeks of captivity and then gradually switched to milk replacer. Two to 3 weeks after arrival, animals were given access to a grassy paddock which had not previously contained animals infected with P. odocoilei. Calves were weaned to a diet of commercial deer pellets, beet pulp and hay at approximately 3 1/2 months-of-age.

First-stage larvae (L1's) of P. odocoilei were collected from faecal pellets of infected mule deer by the Baermann technique (see Fig. 1 for life cycle). Laboratory-reared terrestrial snails (Triodopsis multilineata) were exposed to these larvae. After a minimum of 30 days, snails were digested in pepsin-HCl digest (0.6 g pepsin in 0.7 ml HCl per 100 ml distilled water). Third-stage larvae (L3's) were collected and administered per os to calves. One moose (Mo 12) received 300 L3's while the remaining moose (Mo 10) received 800 L3's. Daily faecal samples were collected and examined for L1's from 40 to 95 and 40 to 98 days post exposure (d. PE) for Mo 12 and Mo 10, respectively. Weekly faecal samples were collected and monitored thereafter until 101 d. PE (Mo 12) and 154 d. PE (Mo 10).

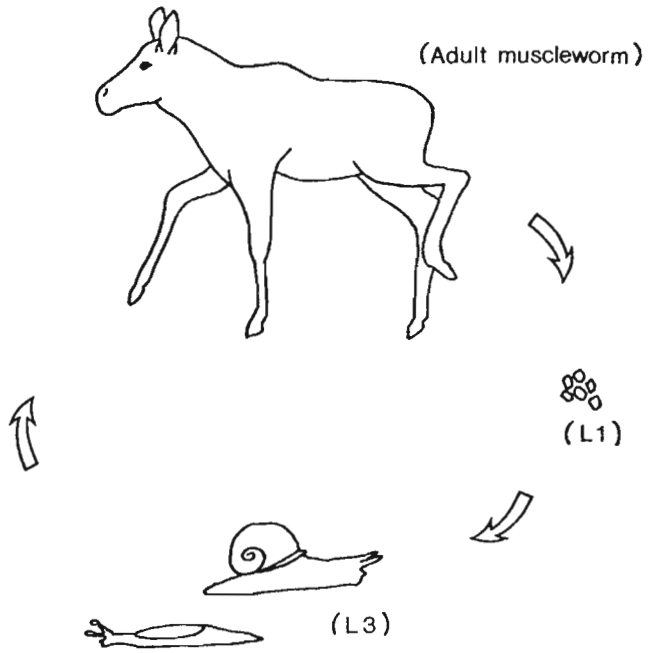

Figure 1. Life cycle of Parelaphostrongylus odocoilei in moose. (Il=first-stage larva, I3=third-stage larva)

Animals were terminated with sodium pentobarbitol injected into the jugular vein. Standard necropsy procedures were used to examine all carcasses. Samples from various organs and tissues were collected and fixed in 10% neutral buffered formalin for histopathologic examination. In addition, skeletal muscles were removed individually. Longissimus dorsi and psoas muscles were dissected at 6X magnification for recovery of worms. Muscles of the hind quarters and abdominal wall were sliced thinly (3-5 mm) and searched for haemorrhages. All haemorrhagic areas were examined at 6X magnification for presence of adult worms. Muscles of the fore quarters were thick-sliced (8-10 mm) and treated as those of the hind quarters. Muscleworms recovered were fixed in hot glycerin alcohol and cleared for examination to glycerine. Tissue samples of haemorrhagic and/or necrotic areas as well as worms in situ were fixed in formalin. Approximately 200 g of lung tissue from Mo 10 was cubed and left overnight in pepsin-HCl digest at 37 C.

Histological preparations were sectioned at 7 micrometers, stained in haematoxylin and eosin and examined for pathologic lesions. Size and number of granulomas and per cent consolidation of lung tissue were determined in each of 10 sites throughout the lungs. Lesions were described with the assistance of Dr. R.J. Lewis, Animal Health Division, Alberta Department of Agriculture.

RESULTS

Both moose developed patent infections of Parelaphostrongylus odocoilei (Table 1) (Fig. 2). No animal showed clinical signs attributable to infection with muscleworm; that is, abnormal stance, movement, respiration or response to touch.

Gross Necropsy

At 101 d.PE, 6 adult P. odocoilei were recovered from the left psoas and left biceps femoris of Mo 12. Worms were associated with localized haemorrhage and usually found in pairs (1♂,1♀). Haemorrhagic tracts and focal haemorrhage were noted throughout various muscles but often worms were not located in the vicinity. Extensive haemorrhage and focal necrosis were noted throughout skeletal muscles of the back, fore and hind quarters of Mo 10. Muscles were soft and pale and fibres separated easily. Haemorrhagic areas varied from 5-15 mm in diam. Necrotic areas varied from small localized areas (1 mm diam.) to much larger areas (10-15 mm diam.). Yellow viscid exudate and/or green caseous material was present in the large areas. Approximately 1/2 of the necrotic areas were associated with haemorrhage and these areas often extended up to 20-30 mm in diam and 80-90 mm in length. Twenty-three adult P. odocoilei were recovered from the psoas, longissimus dorsi and various muscles of the thigh of Mo 10. Worms were usually associated with haemorrhage but never with necrotic areas. As in Mo 12,

Table 1. Infection data for Parelaphostrongylus odocoilei in moose.

	Dose[1]	Prepatent Period (days)	Termination Date[2]	Maximum Weekly Larval Output[3]	Worm Recovery
Moose 10	800	70	217	108.5 (wk. 9)	23 (2.9)[4]
Moose 12	300	67	101	0.2 (wk. 2)	6 (2.0)

1 - number third-stage larvae
2 - days post exposure
3 - larvae/gram faeces
4 - number worms (per cent dose)

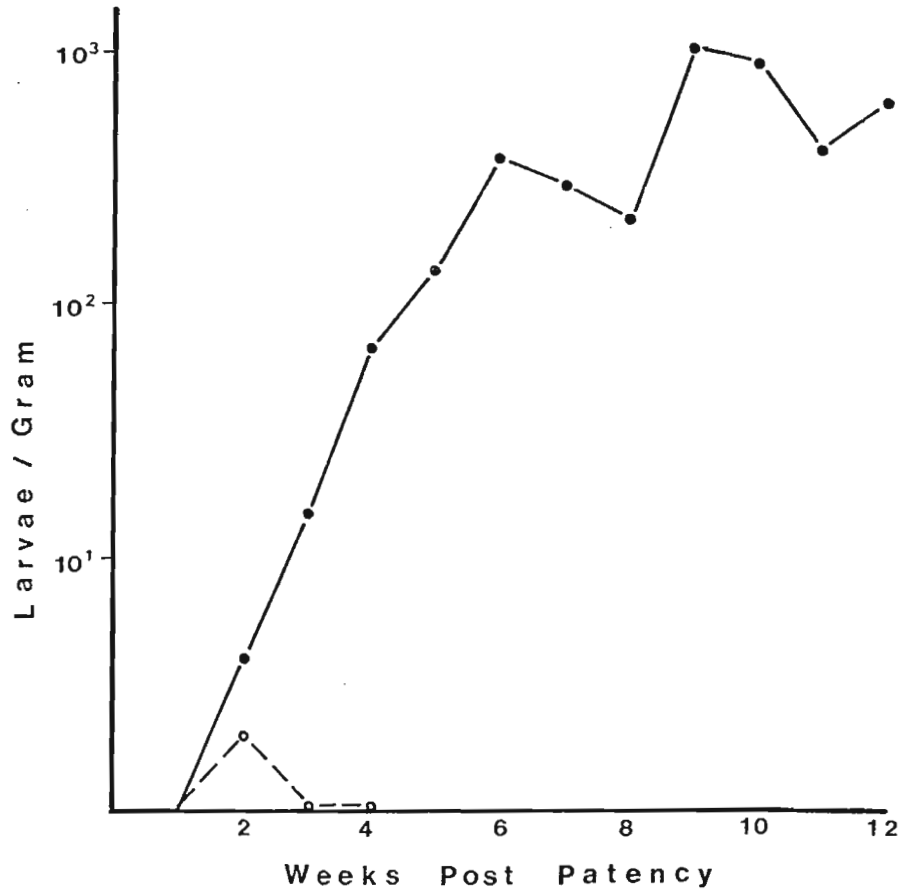

Figure 2. First-stage larvae of Parelaphostrongylus odocoilei in faeces of experimentally-infected moose. Mo 10 (●—●) Mo 12 (o--o)

haemorrhage was often found in the absence of worms. Tissue degeneration involved approximately 10-20% of an infected muscle.

Petechial haemorrhage was present throughout all lobes of the lungs of both moose. Diaphragmatic lobes were slightly darkened but of normal consistency. Cardiac lobes were red-purple and flaccid. Larval recovery from the lungs of Mo 10 was 14.4 larvae/gram.

Histopathology

Adult Parelaphostrongylus odocoilei were found in the perimysium of infected muscles. Worms were occasionally adjacent to blood vascular tracts in the perimysium and the posterior portion of 1 female worm was oriented parallel to a vein and artery. Worms were rarely found between the fibres within a muscle bundle. Adult worms in the perimysium were usually surrounded by a loose connective tissue "coat" with a clear open space between the worm and the connective tissue. This finding was consistent for all worms but may be an artifact of preparation.

Adult worms elicited a consistent response only when found within a muscle bundle. In such cases, a minor buildup of polymorphonuclear cells (neutrophils) and mononuclear cells (macrophages) was seen. Worms in the perimysium rarely were associated with a cellular response.

Large numbers of nematode eggs were located in perimysium as well as within muscle bundles of the back and

thighs. Eggs were found in groups or clumps regardless of the site. Clumps of eggs were never found directly associated with a female worm. Occasionally, "trails" of eggs were found through a number of muscle fibres and across adjacent muscle bundles. Within each group, eggs were usually at a similar stage of development. Most were in the 2-8 cell stage of cellular division; seldom were they beyond the 16 or 32 cell stage.

The presence of eggs in the perimysium or between muscle fibres inevitably resulted in a massive cellular buildup in the area. This response appeared to start in the perimysium and then spread into adjacent muscle bundles. Most of the infiltration was concentrated directly around the eggs. Eggs were often seen enmeshed in a loose fibrous matrix which also contained invading white blood cells. There appeared to be progressive stages in response to the insult and lesions were indicative of chronic progressive myositis. Lesions were similar regardless of muscle involved.

Groups of eggs were extremely large in most cases and the associated cellular buildup often destroyed large (100-200 mm) areas of muscle. The basic organization of the muscle was destroyed throughout large tracts; that is, there were no longer discrete muscle bundles separated by narrow perimysium. Little evidence of repair was noted, even in obviously chronic lesions.

Very few first-stage larvae of P. odocoilei were found

in muscle preparations. When present, they were coiled in loose connective tissue within muscle bundles and surrounded by a loose connective tissue capsule. Within the capsule, numerous giant cells and epithelioid cells were attacking the coiled larva. No evidence of degeneration was noted in the larvae. A diffuse polymorph and mononuclear buildup was seen in surrounding muscle tissue.

Localized focal granulomas in various progressive stages were present throughout all lobes of the lungs. In each animal, size of granuloma and number of granulomas per mm2 were similar regardless of lobe or side of the lung (left vs. right). Size of granulomas was also similar between the 2 animals. However, number of granulomas per mm2 was significantly greater in Mo 10 ($\bar{x}$=0.25) than in Mo 12 ($\bar{x}$=0.05) (t=5.65,d.f.=18). There was slightly more consolidation in cardiac lobes than in diaphragmatic lobes in both animals.

Focal atelectasis and vasculitis were the most prominent lesions in the lungs of Mo 12 . Severe arteritis was noted around a large collapsed bronchial artery in the left cardiac lobe. Collapse and/or occlusion of small arteries was also common in this lobe. Interlobular oedema was present throughout all lobes.

Interstitial pneumonia was evident in Mo 10 as an increase in smooth muscle tissue and thickening of alveolar walls. Response was often centered around focal granulomas containing nematode eggs and larvae. Intensity of response

decreased with increasing distance from the granuloma. Lymphoid tissue was active in all lobes. Atelectasis and vascular collapse were minor insults in this animal. Many bronchioles contained mucous exudate with red and white blood cells present. Lymphoid stasis and venous thrombus were occasionally observed.

Most eggs and larvae observed in the lungs appeared viable. All were associated with a marked cellular response and none was found free in alveolar spaces.

Inguinal, mesenteric and axillary lymph nodes in both moose were hypertrophic with moderately to heavily active germinal centers. Eggs and larvae of P. odocoilei were found in clumps in the subcapsular spaces and medulla of axillary lymph nodes of Mo 10. The subcapsular space was occluded by a granulomatous response characterized by lymphocytes and macrophages. Germinal centers adjacent to eggs and larvae were very active and capped with lymphocytes. Peripheral afferent lymphatics were occluded with eggs and larvae and light pink-staining fluid indicative of oedema was present throughout the medulla of the node. Some eggs had apparently been overcome and subsequently invaded by mononuclear cells. An extensive buildup of mononuclear cells was seen around all free larvae. A few nematode eggs were also found in an axillary lymph node of Mo 12. Response was similar to that described above. Rumenal lymph nodes were inactive in both animals.

The spleen was moderately active in Mo 10 but

relatively inactive in Mo 12. Germinal centers were similar in size in both animals. The spleen in Mo 10 was enlarged and congested with red blood cells, that of Mo 12 was greatly contracted. Neutrophils and haemosiderin were common in spleen samples of both moose.

Within the liver of both animals, extensive oedema and distension of lymphatics was noted in portal triads. Minor mononuclear cellular infiltration was also present in these areas.

Tissue samples were taken from each of the greater and lesser curvature of the fundic region and the greater and lesser curvature of the pyloric region of each abomasum. A first-stage larva was found within the mucosa of the sample from the greater curvature of the pyloric region of Mo 10. A tract of mononuclear cells was present leading from the epithelial border towards the muscularis mucosa. A large accumulation of mononuclears and polymorphs was present anterior to the larva and normal mucosal morphology was destroyed in this area. Lesions were not seen in the abomasum of Mo 12.

To summarize the histopathologic results, it is apparent that eggs and larvae provide the major antigenic stimuli eliciting a marked white blood cell response. Response is concentrated around eggs and larvae but is diffuse throughout large areas of both skeletal muscle and lung tissue. Extensive degeneration and necrosis of muscle fibres occurs in a chronic progressive myositis. The

response starts in the perimysium and spreads into adjacent muscle bundles. As a result, tissue degeneration is not localized but extends throughout infected muscles. Polymorphs and mononuclears attempt to remove debris from haemorrhage and necrotic areas in muscles. Large giant cells are found adjacent to eggs and larvae. Within the lungs, focal granulomas around eggs and larvae are the prominent feature. Extensive atelectasis and interstitial pneumonia are found in lobules containing eggs and larvae. Insult appears to be more severe in cardiac lobes and at the higher dosage level.

DISCUSSION

Large numbers of eggs were seen in the perimysium and within muscle bundles yet few female worms were present. Most eggs were in very early developmental stages, even in chronic lesions. Few first-stage larvae were seen. It appears that eggs are indiscriminantly released as females move through muscle bundles. Development of eggs may be inhibited or delayed in muscle tissue. It is possible that eggs may require oxygen for development (a common requirement in nematodes (Chitwood and Chitwood 1950)) and cannot get sufficient amounts in skeletal muscle. This would suggest much of the reproductive potential of females is lost as eggs are trapped in the muscles.

Eggs apparently provide a persistent antigenic stimulus which is difficult to overcome and elicits a tremendous

cellular response. The result is extensive necrosis and prevention of repair. This would account for the large areas of caseous necrosis and weak muscle tissue noted at necropsy of Mo 10. Similar gross lesions in the skeletal muscle of 1 moose experimentally infected with P. odocoilei were noted by Platt (1978). Nettles and Prestwood (1976) described similar lesions in white-tailed deer infected with P. andersoni. Such lesions were seen only in heavily infected animals receiving 1000 or 5000 larvae. White-tailed deer exposed to lower levels of P. andersoni (5 to 356 L3's) did not show extensive lesions (Nettles and Prestwood 1976, Prestwood and Nettles 1977, Pybus unpub.).

Prepatent periods in the present study were similar to those reported for other moose exposed to P. odocoilei (Platt and Samuel 1978). Number of larvae per gram of faeces was similar at lower doses but much greater at higher doses than those previously reported by Platt and Samuel (1978). In comparison to mule deer given similar numbers of larvae, prepatent period is longer and larval output is much lower in moose (Platt and Samuel 1978, Pybus unpub.).

Nettles and Prestwood (1976) reported abnormal stance and gait, decreased physical strength and reluctance to stand in 2 white-tailed deer exposed to 5000 larvae of P. andersoni. The absence of such signs in moose infected with P. odocoilei (Platt and Samuel 1978, present study) may indicate that muscle lesions are of little consequence to the health of the animal. However, relatively severe damage

was noted in a number of muscles of the back and thighs. Under sustained use or in critical situations faced by free-ranging animals, such damage could have a negative effect on fitness. In addition, duration of egg production by female worms and persistence of eggs and larvae in muscles are unknown. Histological preparations examined in this study indicate that muscle repair is slow in the presence of eggs and larvae. In combination, these factors could put the host at a disadvantage.

Considering the reserve capacity of lungs, it would appear that the insult to the lung would be of minor importance in the overall health of an infected animal.

Necrosis and destruction of muscle tissue is much greater in moose than in mule deer exposed at similar levels of P. odocoilei (Pybus unpub.). However, lesions in the lungs are much less severe in moose. This may be related to the percentage of worms which are able to establish in skeletal muscle and subsequently contribute to egg and larval production. Two to 3% of the initial dose was recovered as adult worms in moose. This compares to approximately 36% recovery from mule deer fawns (Pybus unpub.). Lower production of eggs, accompanied by loss of eggs and larvae trapped or destroyed in the muscles, results in much less insult to the lungs of moose. Thus moose cannot be considered more susceptible to P. odocoilei than mule deer even though worms which successfully mature cause relatively severe damage to skeletal muscles.

There appears to be widespread activation of the lymphoid tissue in moose infected with P. odocoilei. This may be an indication of the strength and/or amount of antigenic stimulus provided by eggs and larvae. Unfortunately, similar histopathologic studies have not yet been completed for P. odocoilei in deer. Regardless of whether this response is higher or lower than that in deer, such a challenge to the immune system could become an important factor in determining how a free-ranging animal responds to additional stresses and insults.

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