The Science Behind Algonquin's Animals

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The Elucidation of the Biology of the Meningeal Worm

*The following is an excerpt from **Research in Parasitology in Algonquin Park** by Roy C. Anderson of the University of Guelph, dealing with **Parelaphostrongylus tenius** (brain worm) that is found in White-tailed Deer and Moose in Algonquin Park. The complete publication (reprint number 6800) can be found in the library at the Algonquin Visitor Centre.*

I had acquired much experience working with the lungworms of mink and this was useful when I commenced to determine the significance of the curious thread-like worms found in the cranium of white-tailed deer in Algonquin Park. I assumed that since *P. tenius*, despite its position in the host, was a lungworm, the first-stage larvae must be shed in the feces of the deer. I collected and examined droppings of deer and found most adults were passing larvae with a dorsal spine on the tail which I assumed belonged to *P. tenius*. I placed these larvae with assorted snails and slugs collected in places where there were no deer. As expected the larvae penetrated into the foot of these gastropods and developed into third-stage infective larvae. Thus, I hypothesized that deer acquired infections when they accidentally ingest snails and slugs crawling on their plant food.

What I needed now was a sufficient numbers of parasite-free deer to test my hypothesis. I do not remember when I heard about the problem the Cyanamide plant in Niagara Falls was having with deer but is was probably from the Department of Land and Forests. The company had fenced in a large area to protect its chemical plant but it inadvertently included a few wild deer in the enclosure. …he (the foreman of the company) would regularly tour the grounds in May and collect the newly dropped fawns. These he would give to zoos who would nurse them to adulthood. The foreman agreed to collect them for me so I visited him regularly and brought the fawns to Toronto where I housed them in my basement.

When about two weeks old the fawns were transported to the Wildlife Research Station and released into a small outside pen. The fawns flourished in the new environment.

…we were busy collecting snails and slugs and examining for larvae the feces of some of the hundreds of deer that lived along Highway 60 in the Park. I placed larvae with my snails and slugs in about three weeks we had plenty of infected gastropods. I used artificial pepsin to remove

infective larvae from them and was ready to orally infect four fawns. I placed infective larvae in milk given to the fawns. To be doubly sure of infections I put some small infected slugs and snails in gelatin capsule and put them in the throats of the fawns.

Then I had to decide when to examine the animals. I had no idea how rapidly the worms would develop but I chose 25, 40, and 50 days for three of the fawns. Each day from 30 days on the feces would be examined for first-stage larvae and I hoped to get the prepatent period by holding one fawn at least until larvae appeared in its feces. This would give me the prepatent period, critical for further experiments with more fawns.

I also had to decide where to concentrate my attention during the necropsy of the fawns. I expected the worms to be very tiny and difficult to find in such a large host. I made the assumption that we should concentrate at the outset on the central nervous system (CNS) because the adults were associated with the CNS and Whitlock had noted worms in the CNS of sheep and I was also aware that *Angiostrongylus cantonensis*, lungworm found in rats, developed in the brain.

On day 25 full of hope my assistant Dave Gibson and I examined the first fawn. We carefully removed the brain and the spinal cord and began systematically taking small pieces of the white fatty tissue and pressing it gently between glass plates we could put under a dissecting microscope. It is difficult to describe our joy when we found our first tiny worm in the tissues of the spinal cord. It is a wonderful thing in science to suddenly get confirmation of something you have suspected, thought about and worked towards. It made all the frequently tedious hard work leading up to a discovery worthwhile. We eventually found eleven worms. The males were only 3.6-7.9 mm in length and the females 4-16 mm in length so our technique had to be reliable. The worms were immature and in the late fourth and early fifth-stage.

On day 40 postinfection we found eight worms. Some worms were in the neural tissue but others were on the duramater. So it was evident that some worms at least leave the neural tissue and move onto the meninges. We were very lucky to have chosen 40 days to examine that fawn. The worms were quite large, all subadults and identified beyond all doubt as *P. tenius*.

On day 50 no immature adult worms were found at all but one free in the subdural space. Two females were in the cranium, the remainder still on the meninges of the spinal cord. This suggested that worms started to move towards the cranium about this time.

The final fawn passed larvae for the first time at 91 days. That too was an important discovery for it gave us information we needed for further experiments. We found in this animal 115 days postinfection 10 female and 5 male worms all in the cranium. Worms had laid their eggs on the dura mater where some had developed into first-stage larvae. Most importantly we found females in the intercavenous sinus, a broad expansion of a vein near the pituitary. In this location, eggs would pass down the venous system, to the jugular and right heart. From there they would be carried with the blood to the left heart and from there to the lungs via the pulmonary arteries. We took pieces of fixed lung tissue and sectioned it for examination with a compound microscope and found exactly what we expected. Eggs were caught up in the fine capillaries between air spaces. They became surrounded by inflammatory cells which formed small nodules. In these nodules the eggs embryonated into first-stage larvae which hatched, broke out of the nodules, entered the air spaces, the bronchioles, bronchi and trachea aided by the flow of mucus moved by ciliated cells to the back of the throat (I like the term bronchial escalator for this wonderful system to clear the lungs of particular matter). The larvae were then swallowed, passed harmlessly through the gut, and out in the feces. Thus, we had a clear understanding of how the life cycle worked and roughly the timing of the various events in the life of this unusual parasite in its host. It was now necessary to use this information to explore in more detail the behaviour of the worms and the reactions of the host to them. Our information did not tell us exactly where the worms were developing in the central nervous system and the pathogenesis associated with the infection.

So in 1964 I again raised fawns and infected snails. I inoculated six fawns and examined them histologically 10, 20, 25, 30, 40 postinfection. One fawn was kept until larvae appeared in the feces. The brain and spinal cord were removed from the animals and preserved in formal saline. Then pieces were taken of the cord at each pair of spinal nerves and between each pair of nerves. Also pieces of tissue were removed from all regions of the brain. These were embedded in paraffin wax and sectioned and stained. The findings were conclusive because of the care we'd taken in setting up the experiment and the great amount of the tissue that was examined histologically.

Worms were in the spinal cord 10 days postinfection and they invaded the dorsal horns of the grey matter where development took place. They remained in this region of the spinal cord until about 40 days when they started to migrate into the subdural space. This agreed well with our earlier experiment. The sixth fawn passed a few larvae 82 days postinfection which is similar to our previous observations.

We studied and described the various larvae found throughout the CNS. The most important observations were that worms elicited little damage in the dorsal horns and secondly, only a small number of worms given reached the CNS to complete their development. This accorded with our early observation. The deer has some way to control the number of worms reaching the CNS. This helps to account for the few clinical signs observed (usually temporary lameness) and the lack of major damage to the CNS. The deer is therefore very well adapted to *P. tenius* and can minimize over infection by mounting a strong immunological response.

Later, in 1966 we heavily infected three fawns and examined them 65, 90 and 144 hours postinfection. Histologic study indicated larvae invaded the stomach (abomasums) wall, entered the peritoneal cavity and followed spinal nerves to the spinal cord. We estimated this journey takes about 10 days. We also noted that deer reacted strongly to the presence of larvae and trapped many in the wall of the abomasums. We deliberately used high doses of larvae to increase the chances of finding small larvae in the tissues. This is unnatural, however, as in the wild deer are generally exposed to very limited number of larvae found in naturally infected gastropods.

Moose sickness

I do not remember exactly when or why I began to think about “moose sickness” that mysterious neurologic affliction of moose in Nova Scotia, New Brunswick, Maine, Minnesota and Ontario which had apparently greatly reduced moose population in some regions of eastern North America. Affected moose lost their fear, were lame, had lumbar weakness, uncoordination (ataxia) and impaired vision. They often walked in circles holding the head to one side and at an abnormal angle. The animals eventually developed paralysis and died.

In 1963, I'd completed preliminary studies of *P. tenius* in deer, studies that showed the neurological involvement of the parasite and its association with deer. I was, however, still interested in filarioid nematodes and encountered some reviews of Japanese work on a filarioid worm normally found in the body cavity of cattle in the Far East. *Setaria digitata*, like most filarial worms, produces tiny larvae which occur in the blood of cattle. When they are ingested by mosquitoes they will invade the tissues of the latter and reach an infective stage which moves to the mouthparts. When the mosquitoes feed again, the larvae leave and can enter the lesion made by the vector and infect other cattle. My interest was sparked by the fact that these larvae, when transferred by mosquitoes to horses, eventually developed in the CNS and produced paralytic disease. This discovery was made by Dr. Chuzaburo Shoho who studied the disease in Japanese cavalry. The neurologic disease in horses was, therefore, the result of a parasite transferred from a well adapted host in which it produced no clinical disease, to a susceptible but unsuitable host in which the parasite behaved in an abnormal manner by migrating into the central nervous system and causing a serious clinical disease. Somehow it occurred to me that perhaps moose sickness was caused by a parasite in the wrong host. It had been speculated for years that the disease was caused by ticks, tick-transmitted bacteria, viruses or cobalt deficiency but none of these explanations satisfactorily explained the basic features and distributions of the disease in moose. I then got together all the reports of each moose and plotted them on a map. The distribution was curious for several reasons. The disease only occurred on the southern part of moose distribution and only in eastern North America. Two exceptions caught my attention and excited me. Moose and wolves had been studied on Isle Royal, Lake Superior for years but there were no reports of sick moose on the island. Similarly, Newfoundland had a well-studied and increasing moose population resulting from the introduction of a few moose earlier in the century. Again, sick moose were not observed there. These two islands had in common that they were devoid of white-tailed deer. And then it was obvious that moose sickness only occurred in places where moose overlapped with white-tailed deer. It was clear to me then that there was every possibility an agent transferred from deer to moose was responsible and it was almost certain to be a neurotropic parasite, namely *Parelaphostrongylus tenius*! If this were true it would solve a mystery that had puzzled scientists for over 50 years.

I approached the Department of Lands and Forests and asked if their field personnel could get me a couple of new born moose calves. As luck would have it Mr. Crichton, the District Forester at Chapleau Ontario obtained two moose calves and had them shipped to Algonquin Park where they were bottle fed and given browse.

Infective larvae were obtained from snails exposed to first-stage larvae recovered from the fawn used in the first study of *P. tenius*. Larvae were placed in milk given to the calves when they were about 3 weeks old. The calves were examined each day for any neurologic signs.

Two to three weeks postinfection both calves became lethargic and seemed unwilling to rise. Weakness and ataxia became progressively more pronounced and terminated in paraplegia. The various signs we observed were exactly the same as those reported in wild moose with “moose sickness”. Examination of the calves nervous system after 40 and 60 days revealed numerous worms in the subdural space and in the spinal cord where they were associated with severe traumatic damage to the dorsal horns of the gray matter. The results showed that experimentally it was possible to produce neurologic signs by infecting moose calves with infective larvae of *P. tenius* from white-tailed deer.

It was now necessary to diagnose *P. tenius* in the cerebral nervous system of sick moose in the wild. Another call went out to the Department of Lands and Forests for the heads and spines of moose exhibiting neurologic signs. In 1965 we obtained the head and spine of three sick moose from the Kenora district of Ontario. Careful histologic examination of the brain and spinal cord permitted us to diagnose the infection in all these animals. Thus, we had proved conclusively that the cause of moose sickness was a neurotropic nematode transferred from deer to moose in areas where the ranges of the two cervids overlapped in North America.

My discovery of the cause of moose sickness when others had failed over a period of years taught me one thing about research. Except for accidents one is most likely to find something if one has some idea what one is looking for. That was a very strong factor on my success. Others had seen worms in eyes of moose, as mentioned above, even in the brain stem of moose but they were looking for something else and never followed up these fortuitous observations which would have required a

great deal of sectioning of the brain and spinal cord. Also, how could they suspect that these slender insignificant nematodes could cripple a large animal like a moose. My background of the behaviour of the parasite in deer prepared me for the discovery.

After I'd discovered the effects of *P. tenius* on moose I had to consider how moose acquire terrestrial snails containing infective larvae. Everyone knows moose browse and I had to find out if they also graze close to the ground where they'd encounter terrestrial snails. I asked several wildlife specialists…invariably I got the reply, "I'm not sure". One evening I was watching a film on T.V. about National Parks in Alberta and there before my eyes was a cow and a calf moose grazing in a field. I was relieved to see that.

My considerations of moose sickness went beyond the diagnosis and clinical and descriptive pathology. I proposed that the main reason moose encountered *P. tenius* in recent times was the expansion of deer range northward in the past 100 years as a result of environmental changes brought about by humans. Forestry practices, fires, and agricultural activities created suitable habitat for deer and they multiplied in regions inhabited by moose.