Walt Shiel interviews
Judith A. Appleton, Ph.D., &
Michael S. Duffy, Ph.D.

Quest for a Meningeal Worm Vaccine

The Alpaca Research Foundation (ARF), in conjunction with other groups in the llama and alpaca communities, provides funding grants to veterinarians and scientists engaged in research that has the potential to improve the health and well-being of our animals. *Alpacas Magazine* is pleased to bring you the second in a series of interviews with the researchers carrying on this important work.

If you own alpacas, you've probably had to deal with worms. If not benign, most worms are at least straightforward to control, and the affected animal usually bounces back quickly.

If you own alpacas in the eastern half of North America, however, you are probably familiar with a potentially lethal parasite, the meningeal worm. Scientifically, it is Parelaphostrongylus tenuis (P. tenuis), but the term meningeal focuses attention on the real problem. These nasty critters migrate into the animal's central nervous system to create neurologic problems in your prized alpaca.

Worse yet, once an alpaca shows obvious signs of infestation, recovery is unlikely. Partial recoveries, probably with residual physical impairment, are possible with extraordinary veterinary measures. Because the signs mimic other neurologic disorders, early detection and positive diagnosis are problematic.

You can reduce the odds of exposure and maintain a careful worming schedule, but with no guarantees of success. Some researchers worry that a too-extensive worming program unintentionally could produce wormer-resistance in other worm parasites.

Thus, the quest of Dr. Judith A. Appleton and Dr. Michael S. Duffy for an effective vaccine becomes crucial. The llama and alpaca communities worked together to fund this important project through contributions made to the Alpaca Research Foundation and the Llama/Alpaca Division of Morris Animal Foundation, with the Greater Appalachian Llama and Alpaca Association the major contributor for the first year.

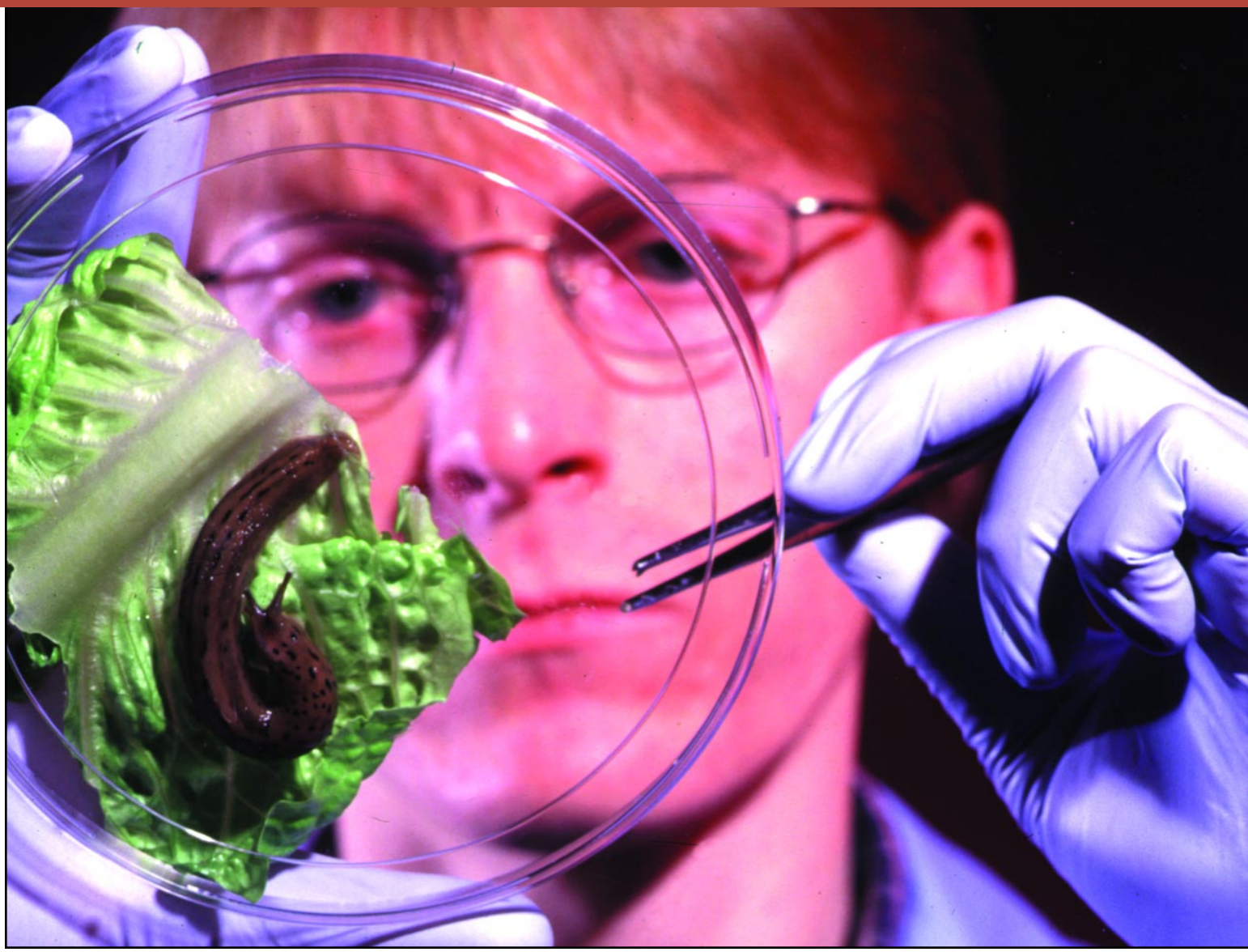
Dr. Appleton is Professor of Immunology and Dr. Duffy is a post-doctoral fellow in the James A. Baker Institute for Animal Health of the College of Veterinary Medicine at Cornell University. Dr. Appleton's background includes many years of research on immunity to parasitic nematodes (worms), while Dr. Duffy has extensive research experience in parasitology and, specifically, P. tenuis infections in deer. I interviewed Drs. Appleton and Duffy on their vaccine research project, which started September 1, 2001.

Walt Shiel: Do you have a particular interest in alpacas or llamas?

Judy Appleton: "I grew up in suburban Chicago and my father traveled extensively in South America… [returning] from one trip with wooden statues of two llamas. They sat on our piano and I used to look at them as I practiced. This was my only 'contact' with llamas or alpacas until I met Gail Fulkerson [Heaven on Dawson Hill farm] and she introduced me to her animals two years ago. They are lovely, graceful creatures."

Mike Duffy: "I am interested in all species that are affected adversely from infection by P. tenuis."

Photo, this page, Dr. Judith Appleton
Photo, facing page, Dr. Michael Duffy with an experimental slug in the lab.



WS: What is your project's primary goal?
JA: "We aim to identify proteins that the parasite, P. tenuis, uses to infect animals. If the alpaca's immune response can be made to interfere with those proteins, then we may be able to make a vaccine that would protect the animal against disease. The challenge is identifying the best proteins to target in the vaccine."
MD: "We have evidence that animals develop an acquired immunity to re-infection… Our present project aims to identify vaccine candidates (proteins from P. tenuis), followed by an assessment of protection conferred by them in vaccination of a laboratory host model (guinea pigs). We presume that protection conferred in a lab host will translate to protection in the target species (camelids)."

WS: How confident are you of finding an effective vaccine?
JA: "We are reasonably confident because at this particular time, there is tremendous progress being made in the vaccine field. The amount of material available from living nematode parasites has always been a limiting factor in studies of immunity and vaccination. This is particularly true for a parasite like P. tenuis that uses an intermediate host (snails and slugs) for development to the infectious stage. Using proteomic and genomic methods is allowing us to identify and isolate proteins with potential use in vaccines."

WS: Why this project at this time?
JA: "My husband raises sheep on our small farm and a few years ago, four of our animals died from P. tenuis infection. This prompted my interest in the problem."
MD: "I spent my Ph.D. working on P. tenuis infections in cervids (deer)… The opportunity to work with Judy on this type of project was really exciting for me so I jumped at the chance to join her lab."

WS: Dr. Appleton, have you considered adding alpacas to your farm?
JA: "We have, indeed. However, we have so many white-tailed deer around our place that I fear for the health of any camelid. Hopefully, we will find a way to protect them!"

The North American white-tailed deer has developed into a natural host for P. tenuis, despite rarely succumbing to the debilitating neurologic disease

itself. Dr. Duffy surmised: "Camelids and other ungulates can succumb to neurologic disease from a single worm. There are undoubtedly anatomical differences, physiological differences, or a combination of both which explain the tolerance or intolerance of hosts to infection."

In deer, the adult worms reside in the blood vessels, sinuses, or dura surrounding the brain where the female worms release their fertilized eggs into the blood stream. These eggs are carried to the lungs and hatch into first-stage larvae (L1). The L1 migrate up the trachea through coughing, are swallowed, and then passed in the feces. The L1 enter intermediate hosts, like snails and slugs, and mature into the infective stage (L3). Ungulates become infected by eating infective L3 while grazing. In alpacas, these L3 may not remain in the dura surrounding the brain but instead migrate throughout the spinal cord and the brain. *(See Figure 1.)*

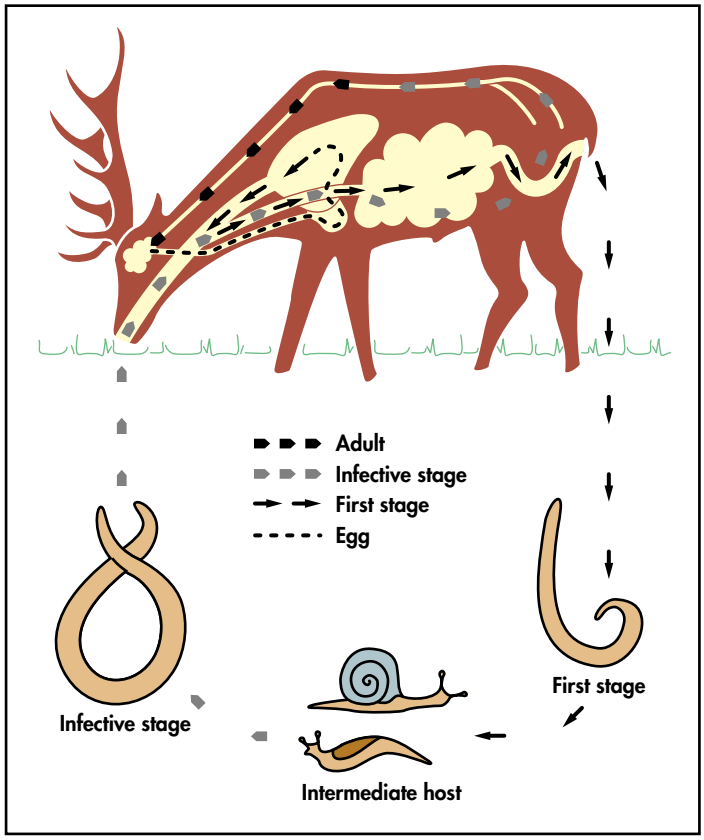


Figure 1. Life cycle of the P. tenuis, modified by Dr. Duffy with permission from Paul C. Mason (Mason, Paul C., 1989, *Elaphostrongylus cervi:* a Review; Surveillance 16, pp. 3-10).

According to Dr. Duffy, "Llamas, alpacas, and other domestic ungulates are 'dead-end' hosts since P. tenuis do not mature to adults and L1 are not passed with feces. The persistence of P. tenuis in tissues of the spinal cord, and eventually the brain, results in physical damage to these tissues and neurologic disease in the host. Signs of infection include a general uncoordination, weakness in one or more limbs, and eventually paralysis involving one or more limbs."

WS: Is P. tenuis a significant problem for alpacas?
MD: "P. tenuis can kill or severely impair llamas and alpacas that become infected. The distribution of P. tenuis is limited by the distribution of white-tailed deer (the definitive host) and snails or slugs (the intermediate host). P. tenuis had been documented only from the eastern half of North America but was recently found in Costa Rica."

WS: Is P. tenuis migration beyond the eastern half of North America possible?
MD: "The low rainfall and low incidence of snails and slugs in the Great Plains area is thought to be a natural barrier. Also, a ban on cervid translocation (in the early 1990s) from eastern to western Canada was based on the possibility of introducing infected deer to the west [that] could 'leap-frog' P. tenuis over a natural ecological barrier with potentially devastating effects."

WS: Why is a positive diagnosis, beyond the observable symptoms, so difficult?
JA: "The worms are very difficult to find [even] on necropsy. It is a very time-consuming task to even attempt it."
MD: "Neurologic symptoms are not unique to P. tenuis infection, so it is possible that other factors are involved. The only method for definitive diagnosis of P. tenuis in any animal is by recovering the infecting worms from the brain or spinal cord. The odds of finding the infecting worm are very low even with a targeted approach, could take up to eight hours, and worm recovery is not guaranteed."

WS: Where and how did you acquire a sufficient quantity of snails, slugs, and infected deer feces?
JA: "When he was a graduate student in New Brunswick, Mike Duffy experimentally infected a white-tailed deer. The deer became infected (but not sick) and shed larvae in her feces. Mike collected the feces and stored them in the freezer. The first-stage larvae from the feces have been used to infect snails in order to produce the infectious L3 needed."
MD: "The L1 are still viable after more than 2 years at -80°C. [Thus accounting for their ability to survive North American winters]. The L1 resume activity in the spring, which corresponds to the emergence of snails and slugs. We maintain a breeding colony [of snails] in Judy's lab at Cornell."

WS: Do the larvae remain viable during repeated winter freeze-thaw cycles?
MD: "[They can survive] in the external environment, although freeze-thaw cycles and desiccation eventually will kill the larvae. The mucous coat that surrounds fecal pellets offers some protection."

WS: Can you describe your project's methodology? Terms like "mRNA" and "cDNA" are pretty esoteric for most of us.
JA: " 'mRNA' is the 'messenger' RNA. It is synthesized using chromosomal DNA as a template. mRNA is 'read' by the cell and translated into protein. cDNA is complementary DNA that is synthesized in the laboratory using mRNA as a template. We make cDNA so that we can identify the messages (different mRNAs) that the parasite produces. DNA is more stable and easier to work with than mRNA. It can be cloned into bacteria so that the bacteria will transcribe the appropriate mRNA and translate that into the parasite protein."
MD: "[mRNA] represents a copy of all the protein-coding genes that are 'turned-on' in a particular life-stage of

P. tenuis. Since each life stage is adapted to survive in a specific site(s) within the host, they likely require a different complement of proteins to enable survival. This difference in protein synthesis may provide a clue as to the relative importance of certain proteins in the process of infection or survival within the host. We then use the [cDNA] for insertion to harmless bacteria or viruses that can maintain the gene until we decide to recover the protein that is encoded by the gene."

JA: "When you prepare a batch of cDNA from the mRNAs in the parasite, you can insert those (clone them) into bacterial or viral 'vectors.' The idea is to get lots of different cDNAs into lots of vectors. Conventional libraries are collections of books with different information or a different story in each one. A cDNA library is a collection of bacteria vectors with a different cDNA in each one. Each one of these cDNAs matches an mRNA from the parasite, so we have created a library from the parasite. Once we make the recombinant proteins from the cDNAs, we immunize rats to make antibodies that we use to characterize the proteins further."

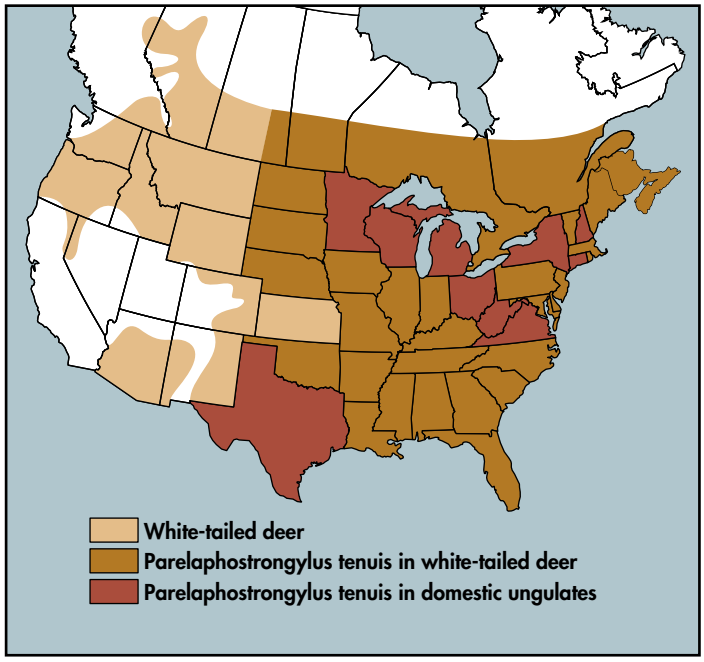


Figure 2. Distribution of P. tenuis in North America, provided by and modified by Dr. Duffy with permission from Natural Resources Canada, Canadian Forest Service, Forest Health Network.

WS: Once you produce a vaccine, how long before it might be commercially available?

JA: "A mass-produced vaccine may be in the distant future (I am being realistic here) because of the small number of llamas and alpacas at risk for disease in this country. Industrial vaccine companies are unlikely to be interested in producing a vaccine for such a limited number of animals. We will try to develop vaccines that would also be effective in goats and sheep (we are seeking support from the USDA to do this work) in order to increase the size of the target population."

WS: Can you recommend effective preventive measures for alpaca owners?

MD: "Since it is impossible to eliminate snails and slugs from pastures, the prevention would have to deal with keeping deer off of pastures that are shared with alpacas and llamas. Fencing would have to be 10-12 feet high to keep out white-tailed deer. In addition, you have to be aware that L1 shed previous to fencing may persist on the pasture and therefore may infect snails or slugs. There is also the possibility of L3 surviving over winter in snails or slugs. It may take several years for infected snails and slugs to be cleared from the pasture.

"Additional considerations for a fenced pasture would include improving drainage of surrounding pastures which may be a source of the L1. Vegetation control outside the perimeter of fenced areas would discourage snails and slugs from migrating in. Eliminating the potential for encounter with P. tenuis would require indoor housing or maintenance of the alpacas in an area free of vegetation."

WS: What about the use of molluscicides to eliminate snails and slugs?

MD: "We investigated molluscicides to keep snails and slugs away from red deer while I was in New Zealand. Copper sulphate was one chemical proven to act as a molluscicide, but it was also highly toxic to ungulates, so we didn't use it. We ended up removing all vegetation from the outdoor pens and spraying to kill all the vegetation around the perimeter."

Meningeal worm is a serious problem for alpaca owners in eastern North America. Infected alpacas die or may survive with serious neurologic disorders. Despite evidence of cures, available research indicates small numbers highly dependent on the uncertainty of early diagnoses. While awaiting an effective vaccine, your best bet is a solid worming program (concentrating on the late spring to early fall period of prime susceptibility) and minimizing pasture-sharing with snails, slugs, and white-tailed deer.

According to the Alpaca Registry, which has collected strictly voluntary cause-of-death data from owners only since 2001, about three percent of the reported deaths are due to P. tenuis. Approximately 60 percent of the 42,000+ registered alpacas in North America reside in known P. tenuis affected regions. *(See Figure 2.)*

When a vaccine becomes available, alpaca owners may need to band together with owners of other affected ungulates to convince the drug manufacturers that this much-needed vaccine can be profitable.

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Walt Shiel has written dozens of articles for US and overseas aviation magazines and a popular comprehensive history book of military Cessna aircraft, Cessna Warbirds. *He and his artist wife of 34 years are considering adding alpacas to their Texas mini-ranch's four horses, dogs, cats, and itinerant wildlife. Walt can be reached at www.WaltShiel.com, Walt@CessnaWarbirds.com, or (817) 596-4319.*