

A Snotty Situation: **Investigating Sinus Tumors in Rocky Mountain Bighorn Sheep**

by Karen Fox
DVM, DACVP

As a veterinary pathologist, I spend much of my time doing animal autopsies. Yep, I am really good with a cleaver. When this topic comes up at holiday parties, it has the strange ability to remind people of things that urgently need attention in the other room... but truly, my job is fascinating. Not only do I get to try to solve a mystery with each case, I'm also put in a unique situation where I can contribute to management decisions. My job, detecting and studying diseases in dead animals and tissues, can sometimes be the key to understanding population fluctuations and managing populations effectively. This is why I am often given the chance to examine wildlife that have been found dead or euthanized, and it is how I came to autopsy several bighorn sheep where I discovered a sinus tumor never described before in this species.

SINUS TUMOR UNDER MICROSCOPE: The wavy light-pink wisps of cells, dotted with blue nuclei, make up the soft tissue tumor which has replaced what used to be solid bone. All that remains of the bone are the bright pink islands with jagged edges, where the bone continues to be eaten away.



Nasal secretions, mucus, nasal exudates, snotsicles, mucous exudates—who would have thought that snot has so many names? Or, that this humble substance has so much to reveal about the present and future health of bighorn sheep populations.

SHEEP SNOTSICLE: A bighorn sheep ewe with nasal discharge may be harboring a sinus tumor.

PHOTO COURTESY OF KIM WOODRUFF, COLORADO PARKS AND WILDLIFE

Cues From a Ewe

It started with an old ewe that had been euthanized because of suspected respiratory disease. The ewe showed general signs of old age and had failed to successfully raise lambs for several years. The local wildlife managers and biologist had also noticed she had a snotty nose and suspected pneumonia. The carcass came to me for an autopsy to investigate the cause of illness. When I examined her, I was surprised to find her lungs in pretty good shape for an old bighorn. There were signs she had fought pneumonia a long time ago, but not much actively going on. Then I took a closer look at her head. A few cuts with the band saw revealed that she had mucus filling her nose and all of her sinuses. And there was solid tissue filling some of the sinuses, too. This was something new, and I was eager for a look under the microscope.

Back at the lab, the tissues were embedded in paraffin wax blocks, cut into very thin sections, mounted on microscope slides, and stained for evaluation. Under the microscope, I was able to see individual cells and details that provided more information about what was going on. I was surprised to find that the tissue filling the sinuses was actually a tumor! This finding became even more

interesting as I started searching for, and finding, similar tumors in more and more bighorns. It was interesting because tumors are uncommon in wildlife, and when they do occur in multiple animals, it's often because of something infectious, usually a virus.

How does a virus cause a tumor? It depends a lot on what the virus is, but once a virus infects a cell it may interfere with the cell's normal growth signals—either by turning on too much of a normal cell growth promoter or turning off normal growth inhibitors. The cells multiply uncontrollably, and a tumor results. You may be wondering why a virus would evolve mechanisms to cause a tumor. In the case of the bighorn sheep sinus tumors, we can speculate why. We assume that the virus is spread when sheep sneeze, similar to how the human cold and flu viruses are spread. And because respiratory viruses are usually found in nasal secretions (snot), it makes sense that the virus would benefit from making lots of secretions. What better way to spread your own kind than making a snot-producing tumor?

Thus was born my hypothesis: That sinus tumors in bighorn sheep are caused by a virus that spreads infectiously through nasal secretions from one sheep to another.

Seeking Answers Through Experimentation

With my hypothesis in hand, I sought the nearest virology lab that was researching tumors caused by viruses in wildlife. This sounds pretty specialized, and it is. But luckily, just such a lab exists at Colorado State University (CSU). Also lucky for me, the virologist was interested in my discovery and took me on as a graduate student to further investigate this disease. Together, we started our search for a virus. I had a few viruses in mind based on diseases I knew about in domestic sheep and goats, but all of our initial tests for those diseases were negative. This was going to be harder than I thought.

Our next step was to determine if the disease was indeed infectious. Tumors have other origins that could be involved, such as exposure to toxins and genetic causes, and we wanted to test our hypothesis that this disease was infectious and transmissible from one animal to another. We designed an experiment to infect bighorn and domestic sheep lambs with an inoculum made from a naturally-occurring tumor and the associated mucous exudates (snot). I am fortunate to work closely with Colorado Parks and Wildlife (CPW). They provided facilities and



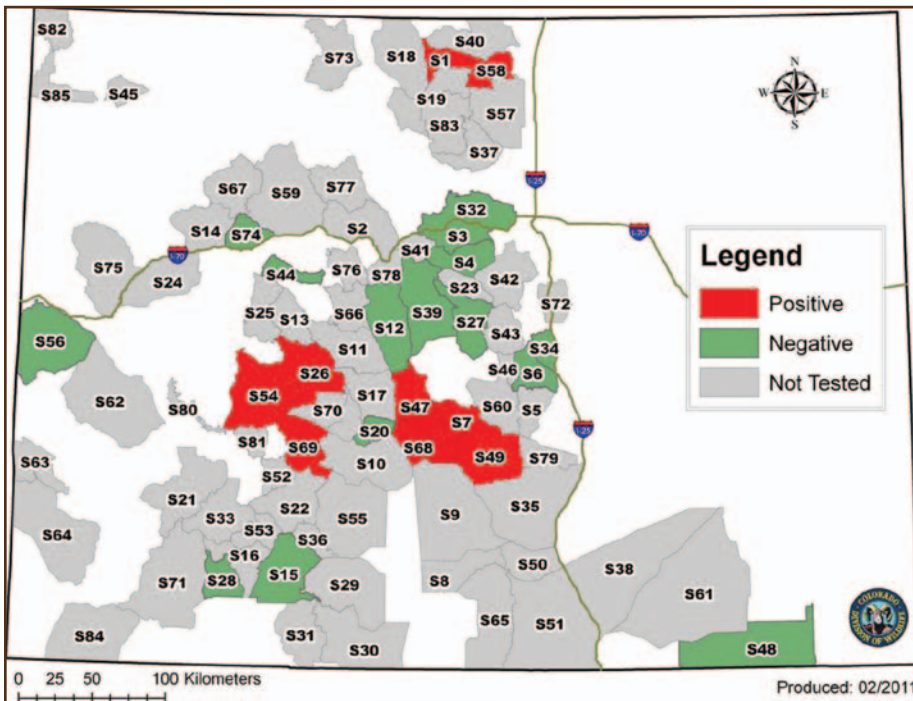
animals for the experiment and funded this project, along with contributions from the Boone and Crockett Club, the CPW Sheep and Goat Auction and Raffle fund, and the CSU College of Veterinary Medicine and Biomedical Sciences.

In 2010 we infected the bighorn and domestic lambs by putting tumor inoculum into their noses. Because we were still unsure of what causes the tumors, we could only hope that the inoculum contained tumor-causing virus at concentrations great enough to cause an infection and a tumor. After a year and a half of waiting, the experiment is now nearly completed, and soon we will know if we have successfully transmitted the tumors. We can only definitively diagnose a tumor by removing the tissue at autopsy and examining that tissue microscopically. However, during the study we have been monitoring the sheep using computed tomography (CAT scans) of their skulls. Twice during the study we have transported the lambs to the CSU veterinary hospital where they are sedated and scanned



TOP: SINUS TUMOR AT AUTOPSY – The sinus of the forehead of this young ram is filled on one side with a sinus tumor. The unaffected side shows the normal delicate bone structures that have been destroyed on the other side by the tumor. **MIDDLE: CAT SCAN –** A sedated bighorn sheep, part of our experimental transmission study, is being loaded into a CAT-scan machine for evaluation for sinus tumors.

The blindfold helps decrease visual stimuli to reduce stress as the animal enters and comes out of sedation. **LEFT: Distribution of bighorn sheep sinus tumors from cases diagnosed in Colorado.** Red areas identify where sheep with tumors have been observed; green areas are where sheep have been examined but no tumors identified; and gray areas are those where sheep have not been examined.



DO YOU WANT TO HELP THIS STUDY?

Hunters and taxidermists who are interested in contributing bighorn sheep skulls can contact their local wildlife agency and ask whether the agency is participating in this research.

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with a CAT scan machine similar to one you might encounter at a human hospital. The CAT scan produces cross-sectional images of the head which we then analyze for any changes in the tissue and bone of the skulls. This information will help us evaluate when and if tumors develop, and how they change over time.

While waiting for the final results of our experimental transmission study, we continue working in the lab to identify specifically what infectious agent is causing the sinus tumors. We still suspect a virus, and so far we have found at least two viruses in the tumors. We are working to determine if either of these viruses actually is causing the tumors, using samples submitted from wild bighorn sheep.

Help From the Hunting Community

Many of the bighorn sheep samples we examine have come from hunters cooperating through their taxidermists. These hunters have chosen to donate the unusable portion of their bighorn sheep skulls after taxidermy is completed, and participating taxidermists have agreed to collect and hold the skulls until they can be transported to our lab for examination.

For taxidermy the horns, skull cap, and skin are removed from the skull, and typically the leftover skull is discarded. We can use that discarded portion of the skull to look for tumors in the sinuses located above the upper teeth. In addition, we may also find tumors in the forehead and extending up into the horns, which makes it best to analyze the entire skull if it is available. Samples of whole skulls have come from sheep hit by cars, euthanized individuals, or animals found and retrieved—sometimes through extraordinary efforts—by Colorado Parks and Wildlife personnel.

Using all of the samples gained through a truly team effort, we have furthered our laboratory research and also constructed a map of locations where bighorn sheep with tumors have been identified in Colorado. Examination of over 100 bighorn sheep heads has yielded evidence of tumors in approximately one third of those examined, with some indication that cases are clustered geographically. There remain many geographic areas still to be sampled, but the preliminary observation of geographic clustering is consistent with our hypothesis that bighorn sheep sinus tumors are caused by an infectious agent being spread within certain herds.

The Pneumonia Puzzle

People often ask me whether or not sinus



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tumors have anything to do with bighorn sheep pneumonia. As most readers of *Fair Chase* will know, pneumonia has caused problems for bighorn sheep in western North America for well over a century. When an outbreak occurs in a herd, many of the sheep die and the survivors seem to somehow carry the disease and pass it on to lambs born in the spring. The lambs rarely survive when they develop pneumonia, and this lamb mortality may continue for years after the initial outbreak in the herd. Exactly how the lambs continue to get sick year after year remains poorly understood, but efforts to grow populations become very difficult when many lambs never make it to breeding age.

With this in mind, you can understand why researchers would be very interested in any additional piece to this confusing disease puzzle. Unfortunately, at this point we don't know if the sinus tumors contribute to other bighorn sheep respiratory diseases. We do know that we have found the tumors in herds of sheep that also have struggled with pneumonia. We also know that the bacteria that can cause pneumonia can also be found growing in the pools of mucus that are found in sheep with sinus tumors. Hopefully, as we learn more about both diseases, we can make better sense of how they may or may

not interact.

Where do bighorn sheep sinus tumors come from? How are they spread in sheep populations? And what, if anything, does this have to do with pneumonia? These pieces of the puzzle remain unknown. We have a great deal more to learn about these diseases, and while we are close to answering some basic questions about bighorn sheep sinus tumors, the questions outnumber the answers for now. Fortunately, an ever-growing and very necessary network of people throughout the United States and Canada is helping to collect samples, provide insights, and collaborate on research to find answers to these questions.

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Karen Fox is a veterinary pathologist working to complete a Ph.D. in the Department of Microbiology, Immunology and Pathology at Colorado State University. Her study on paranasal sinus tumors in bighorn sheep got an initial boost from a Boone & Crockett Conservation Research Grant.