Part I—Body Count

"I'm glad you're here, doc. This is really starting to get spooky...."

It was a hot August afternoon and Bill was standing in a field at the edge of a woodland on his farm. He had about 200 acres in northwestern Minnesota, including some woods, a couple of fields he planted in corn and soybeans, and a small pond.

Bill was talking to Carl Spackler, a veterinary pathologist, one of the few in the country. Carl had been the veterinary pathologist for the state for about 10 years, and he had never seen anything like this before.

It hadn't rained in about two weeks, and the air was hot and still. The stench rising from the deer carcass that Bill and Carl were standing over was starting to get pretty strong. A dead deer wouldn't be enough to bring Carl out to the farm, but the dozen or so animal deaths that had occurred on the farm within the last week was of concern.

"So tell me what's been going on, Bill."

"Well, last Monday I found a couple of dead ducks at the edge of the pond. Then Tuesday a deer in the soybean field. Thursday it was a coyote at the edge of the woods. To be honest, I didn't mind the dead coyote too much—with chickens around, the fewer coyotes the better. But it's still strange. Saturday was bad—another couple of ducks, a couple of raccoons, and a deer. That's when I called the state. I think someone is poisoning my land. This deer here is fresh from this morning. It's not like any of them were shot or anything, so I don't think it's a poacher or a jerk shooting animals for kicks. But I'll tell you, doc, this is kind of weird."

Carl had to admit he was a little puzzled. The animals were different kinds of vertebrates and they had all been found dead in the same area within a week.

Taking a step back from the stinking carcass, Bill wished he had been able to get here earlier in the day. For one thing, it might have been a little cooler, but mainly because the deer carcass would have been a little fresher. Judging by the smell and the level of rigor mortis, the deer had probably been dead for about 12 to 18 hours, meaning it died some time last night. And that meant Carl would have to work fast.

Lucky for Carl, Lauren, a first year veterinary pathology resident, was with him. She had about three years experience as a vet, but had wanted a bigger challenge and so had decided to try veterinary pathology. She was a vet in the Twin Cities, and didn't have too much experience with "non-traditional" animals.

It would be a challenging case for both of them. But where to start?

Your Task

List possible reasons the animals may have died. Your reasons can be stated as testable hypotheses, along with data supporting the hypotheses.



Part II—Seeking a Pattern

Carl and Lauren hoisted the dead deer into the refrigerated box on their truck and then picked up the rest of the dead animals scattered over Bill's farm. Carl noticed that other than decomposition, the animals all looked in pretty good shape—they weren't skinny or gaunt. He proceeded to take some soil samples from near where the animals were found.

"Do you think they were poisoned?" asked Lauren.

"I don't think their food was poisoned, because they all have different food sources—deer eat leaves and grains, ducks typically feed in the water, raccoons are omnivorous, and coyotes are scavengers," replied Carl.

Lauren knew that even if the animals were poisoned, they would need some clues on what type of poison to look for and in what body tissues. You can't just test an animal for every possible poison.

Carl said, "Let's look around, though I have a hunch. Poisoning is actually at the top of my list right now."

Lauren wondered out loud what made Carl think that the animals might have been poisoned, even after he said he didn't think it was in their food. Lauren remembered hearing something about naturally occurring anthrax being found in some soils... but then how would ducks fit into that?

Carl gave a garbled response about some patterns and about climate. Lauren was curious.

Carl and Lauren took a walk around the property and took notes. Along the way, Carl and Lauren discussed data they might want to gather. Lauren was curious about the direction Carl was going in. First, Carl told Lauren they should get climate data for the last few weeks, specifically rainfall and wind. As they walked around, Carl mentioned to Lauren that Bill had said the only water source was a pond, and that's where they were headed.

The pond wasn't much to look at. Water levels appeared to have dropped, and there was no stream inlet. It looked like the water had been stagnant for a while, and there was a surface film at one end of the pond. They took some water samples and headed back to the truck.

They stopped off at the farmhouse to see Bill. "Well, I've got a strong hunch I know what the problem is. We'll know for certain within a few days. In the meantime, sit tight and let us know if any more dead animals pop up," Carl told Bill.

"Do you think someone has been poisoning the water?" asked Bill.

"No, not exactly. I can't say more on just a hunch, but I'll bet a Philly cheesesteak that I'm right. You'll hear from us by the end of the week," replied Carl.

By now Lauren was very curious. As they were getting into the truck, she asked, "So what do you think, Carl?"

Carl smiled and said, "No, what do you think?"

"OK," Lauren said. "No external wounds, so they weren't shot. There aren't many infectious diseases that can cross species boundaries like that, and there haven't been reports from other areas. So it sounds like it's something very local. They probably ingested something—probably not food, because they eat different things. Maybe water?"

"Alright," said Carl. "It could be someone dumping something into the water to poison animals, but that's pretty conspiracy-theory. It might be something seeping in, but I don't think there's ever been industry

around here, there's no pesticide storage around, and I don't think the pond is spring fed. So if there is something in the water, it's being produced in the water. Let's change gears. What do you know about how toxins can act?"

Lauren was getting to the limits of her knowledge here. Even though as a vet she dealt with a few dog and cat poisonings, the owner always knew what the substance was, which made treatment easy.

- "Uhhh, well, there are hepatotoxins, neurotoxins, toxins that act on cell membranes.... Some can act over a lifetime, some act almost immediately."
- "OK, good start. About the time-scale.... Do you think these animals were affected for many weeks, days, or pretty quickly?"
- "Uhhhh, I don't know. Wait. As we put the animals in the truck, they seemed in really good shape. The raccoon was fat and the deer looked in really good condition. They probably weren't sick for long, so I would say it was quick. Plus, Bill didn't find any dying animals, just dead ones. That also suggests a quick death."
- "Excellent, Lauren. So what kind of toxin might act quickly?"

"Yep, that's what I'm thinking too. So to understand how neurotoxins work, we need to know how the nervous system works—anatomy and physiology. Let's meet tomorrow for a review of the nervous system."

Lauren sensed she had some work to do. When she got home that night, she pulled out her Anatomy and Physiology texts, and made an outline of the things she needed to know.

Ouestions

- 1. How does information travel along a neuron?
- 2. How does information travel between neurons?
- 3. How does information travel between neurons and other cells?

[&]quot;Neurotoxins can act fast."



Part III—Neurotoxin?

With a better understanding of how the nervous system works, Lauren was ready to meet again with Carl. After reviewing the anatomy and physiology, Carl said, "As you've probably guessed, the hunch I had was that a neurotoxin is poisoning the animals. Because the animals are found in different habitats and occupy different places on the food chain, I'm guessing the neurotoxin is found in something that is common to all of them—their water source."

"But yesterday you said that it's probably not something that's seeping in nor something someone deliberately put there," Lauren said.

"Right, I think it's a neurotoxin that is being produced in the water by microorganisms—cyanobacteria to be exact. Cyanobacteria blooms can occur in any kind of water, but can especially be found in stagnant water, and can appear as a surface film at one end of a lake or pond. The cyanos produce toxic metabolic byproducts, including some powerful neurotoxins. I noticed a surface film at Bill's pond yesterday. It hasn't rained in a while and the air's been pretty still, so the film of cyanos has had time to accumulate."

"OK, so how do these neurotoxins work?" asked Lauren.

"One kind of neurotoxin is called Anatoxin-A," replied Carl. "It works by mimicking acetylcholine, but the body's natural acetylcholinesterase can't break it down. So what would that cause, Lauren?"

Lauren thought for a moment and then responded.

"Excellent," Carl said. "Another kind of neurotoxin is Saxitoxin, which acts by blocking the voltagedependent sodium channel. And what would that cause, Lauren?"

Again Lauren answered.

"Excellent again," said Carl.

Then Lauren asked, "So how do you know for sure it's either one of those, and if it is, which one is it and what can you do about it?"

"We took water samples," Carl said, "so we can look for the presence of the cyanobacteria in the water, and we can use high performance liquid chromatography to look for either of the suspected toxins. Since we know exactly what we're looking for, we'll know if it's there or not. Finally, we can test the stomach contents of at least the recent kills for presence of the toxins. As for what can be done, Bill can fence the area off to keep animals from drinking from the pond. We can come out and test the water samples whenever the weather gets like this, and if it's a recurring problem, Bill can add an algaecide to kill the cyanobacteria. For right now, it's supposed to cool down and rain for a couple of days, so that will take care of the problem, at least for the next week or so."

Questions

- 1. How do the actions of each neurotoxin act to block nervous stimulation of the muscular system?
- 2. What is rigor mortis, and how would the onset of rigor mortis differ depending on the kind of neurotoxin the animals were exposed to?

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