LEUKOENCEPHALOMALACIA IN BLACK RHINOCEROS

Dave Kenny

TAPE 7A 642

Kenny: The disease entity that I was going to discuss is leukoencephalomalacia, it is not really a disease that I was particularly interested in; but when 50% of the cases occurred at your zoo, the director is interested in you starting today. So I have had to learn about it. I titled my talk leukoencephalomalacia, as Eric [Miller] alluded to yesterday, and this case also confirmed that. Dr. de Lahunta reviewed this brain also and felt it was a leukodistrophy, and that the other tissues that were affected were mostly because of the extension of the inflammatory process and necrosis.

Just a quick review of the cases. I am going to spend most of my time on the most recent case. It is a little bit different and I think everybody got a copy of the other three case's publication that Eric Miller had done. The first case was in 1979 at the Denver Zoo, it was a two month old female. I would like you to note that all the known cases have been females, and they are relatively young animals. The oldest being this two year old. The most recent case died when it was euthanized at 16 months of age, but it's onset of clinical signs occurred at three weeks.

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That is what made this one a lot different, is that it survived for 15 months. I will also add that in 1978, Denver Zoo had another female by the same pair that produced this first one. It was found in the moat one morning, it was a six month old female. It was presumed that it had drowned. In retrospect we really wonder if this could have been another case, because the brain was not harvested. If you have ever had to take one, it is like breaking through a cinder block wall to get a walnut out! I was interested that Nancy [Kock] said that some wild rhino brains had been taken. Were any of them particularly young animals, or were they all adults?

N. Kock: Both actually. I know it is hard, it is not easy. I always make my assistant do it!

Kenny: So I am going to talk about an animal that was born October 4, 1990. Her name was "*Quanza.*" We routinely do a neonatal exam on the rhinos on day two. I will discuss that a little more later. For the first three weeks, although she seemed kind of small and a little weak for the first three days, she seemed to progress relatively normally. It did take her a little more extra time to learn how to get up and over the curb as she tried to track her mother back and forth along the pad. But still there was really no evidence at this time that there was anything wrong with her. As I mentioned we try to capture them, which is always an exciting experience, on day two. We weigh them, take blood samples, give them antibiotics, and treat the umbilicus. Just a comparison of some weights... We have had 13 babies born at the Denver Zoo. We could not do neonatal exams until Dr. Cambre arrived. At first I wondered if there was something with the weight. She was the smallest rhino ever born at the zoo. On the other hand, the one that occurred

in 1979 was the biggest rhino calf that we ever had. So they have ranged all the way from 66 to 113 pounds. "*Tony*" is the most recent baby and he is a male baby from the same parents as "*Quanza.*"

Munson: Dave, I would like to interject that growth retarded babies are very susceptible to things like *septohematomas*, much more so. 026

Kenny: We wonder if she was a preemie. We thought that her gestation was about 15 months. As far as we knew parturition went relatively smoothly, one minute no baby and an hour later, there it was. It seemed to have happened pretty quickly. At three weeks of age she became acutely ill. The morning keeper at eight o'clock came up to the office and said there was a serious problem. Just seven hours before, the night keeper had seen her playing with her mother and acting relatively normally. So it happened really quick. I will go over some of the signs. She became very depressed. What we saw was that she was continuously vocalizing. She had a left sided head tilt which she had throughout her whole life, circled to the left; head pressing, hypermetric, salvation, lip smacking, and she appeared to be blind, she would bump into walls. We pulled her again from her mother at this time, did an examination and took a lot of samples. We gave her some i.v. fluids at this point. We put her back with her mother in the hopes that maybe she would start nursing. The next day we had to remove her. The continuous vocalization seemed to upset her mother and she started to traumatize it and push it around the stall. We had to take it to the hospital at that point.

Treatment I do not think it certainly had any effect on the disease itself; but I think this is probably what got us through the initial acute stage, and perhaps why she survived so long afterwards. Basically it was just the "kitchen sink" of supportive therapy. We talked to an equine neonatal foal unit and they recommended using metoclopramide to speed up gastric emptying and promote some defecation. She seemed to be constipated at the time. I discontinued that after about three days, she seemed to be getting more and more depressed. I looked at the package insert for Reglan, and they said a certain percentage of human patients become suicidally depressed on the drug. I thought the animal was dying. I took her off, and the next morning she was much brighter and alert. So, it is just my impression, but I would not recommend the use of Reglan in a rhinoceros. We used Carafate and ranitidine for gastric ulcerations. We had to tube feed her five times a day for about three weeks until she would finally take a bottle. So we also have had our share of intensive efforts with trying to keep a rhinoceros alive. I think for the first three weeks we were there every night until midnight or one o'clock doing these treatments and feedings. It took a lot of people in the beginning just to hold her down, even though she was quite sick.

We had the opportunity to do a lot of diagnostics over a 15 month period. We will try to go through each one of these. We drew over 25 blood samples during this period of time, mostly with just manual restraint, kind of the same technique that Eric [Miller] described. Hematology I do not think was extremely helpful, especially when you see the severity of the lesions later on. Certainly at the initial blood sample there was an elevated white cell count, neutrophilia with a left shift, a lymphopenia, and probably

monocytosis. So some indication of perhaps some necrosis going on. The mean represents over 18 white blood [cell] samples. The normals I took from ISIS 1992 on black rhinoceros, male/females less than a year of age. I forget how many numbers went into that. But basically it was pretty close to that most of the time. Clinical chemistries... I suppose I could have highlighted glucose. There was one sample that was 140, I think Eric [Miller] reported a hyperglycemia in some of these rhinos. Alkaline phosphatase in the other rhinos was also elevated, it was the same here. Again, there was really nothing eye popping in any of these chemistries or hematologies over this entire period.

We had a veterinary neurologist come in and evaluate her. [She had] ventral strabismus. She seemed to have a downward fixated gaze, and even when he forced her head up, she would continue to look at the floor. He said in small animals sometimes this can be a sign of hydrocephalus. He recommended we do a CSF tap and CT scans, which we did.

Over the course of events she never had normal stools, they were always kind of liquid to semi-firm. We collected 26 fecal cultures, 62% were positive for *Salmonella*. Actually the first positive we got was from a stomach aspirate. Before we tube fed her we aspirated back some of the contents, and that was the first *Salmonella* we got back, *enteritidis* Group B serotype *derby*. I do not really think it had anything to do with her disease, but it was a compromised animal and susceptible to it. She never really showed any serious clinical signs that she was sick with *Salmonella*, but she seemed to be a constant shedder.

I can not discuss rhinos I guess without vitamin E. On neonatal exam there was no detectable levels of vitamin E. I was interested when Rob [Stuart] said that when dairy cows are about to give birth, their levels drop, I was wondering if that was also reflected in the calves. One of the other animals from Kansas City that had leukoencephalomalacia also had very low levels; although the one that Eric [Miller] had had very high levels for a calf, probably the highest that had been recorded. We routinely give them [vitamin] E. At that time we were doing it with Bo-Se, and so at three weeks her level had risen to what Ellen [Dierenfeld] has reported I think for free-ranging animals. So how this fits in, I guess I really do not know.

We did not think she had leptospirosis, but we had so much serum in the bank, so we decided we should do something with it. We sent off an initial acute sample the day of the attack, and sent another one five months later. They were all negative [(*canicola, grippotyphosa, hardjo, icterohaemorragiae, pomona*)] We then vaccinated her with a six way vaccine, and she did show a response to the vaccine.

We did blood pneumonia and bile acids several times. I used an equine normal for blood pneumonia. Some of the symptomatologies seemed to appear like a hepatic encephalopathy to us, so we wanted to pursue that. I think we had a false hope that we could prove it. In some instances that can be surgically corrected, and we were willing to try that. Blood pneumonia has to be packed in ice right away and rushed to the lab within 30 minutes. But compared to the horse it seemed like she had relatively normal levels. Bile acids at first seemed to be elevated. We took some other samples out of the serum bank, two adult and two juveniles. They came up with 9.4 mol/L. When we did two other neonatal samples from

the serum bank, those levels were much closer to what this animal showed. Again, I am not really sure whether this means anything or not.

We did bring her up to Colorado State University three times. She had three CSF taps, lumbar taps. The last one was at postmortem. In this case it was relatively not helpful. The protein level was not very high [(21.3 mg/dl)]. I think one of the ones that Eric [Miller] reviewed in the paper did have a high protein. This one was also extremely acellular. So again, not very helpful for diagnostics.

Anesthetizing her was not really that hard. I do not know if it was part of her disease, but she seemed to like to stick her head in buckets and garbage cans. You would find her escaped from her pen with a garbage can on her head and bumping into walls, so she liked to put it into the... We stole a traffic cone from down the street and we cut off the end. All you would have to do is hold it out and she would walk right in there. So basically for anesthesia we masked her down this way, and then either intubated her or kept her on a mask. Here you can see her masked down with the traffic cone, and an anthill worth of people getting ready to intubate her. The anesthesiologist said it was like intubating a bulldog. There was so much pharyngeal tissue that just fell in front of the glottis, that it was pretty difficult to do; but actually they got better at it by the third time.

Going into surgery we wanted to pursue the diagnosis of portacaval shunt. So we were going to take a liver biopsy and go ahead and catheterize her and try and do a contrast portography. They took the liver biopsy using an argon laser. The sample came back as mild diffuse hepatocellular swelling that was considered really not very significant. We were unable to elevate the jejunum out of the abdomen. I do not know if anybody else knows much about rhinoceros anatomy, but this is common. It seemed to be attached to the dorsal body wall, and we were not able to extract it through the incision. So we pulled her spleen out and catheterized one of these veins in her spleen in order to do the contrast study. After it was catheterized, we went ahead and closed up the incision and just left the catheter out. Then we took her to radiology, injected the die, and took a radiographic every second for nine seconds to try and see if she had a normal hepatic vasculature. She handled all of these anesthetic episodes really well, which surprised me with how sick she was. That did not work out, she had normal vasculature.

We also did three CT scans, two conventional and one dye enhanced CT scan. It takes about an hour an a half with this particular scanner to do it and the animal can not move through the whole hour and a half, so she has to be deeply anesthetized. There are new circular CT scanners that can do some of these things in less than a minute, but we really did not have access to that. We were also interested in trying an MRI, but we ran into some political problems. We were going to do it with Children's Hospital, but when she started shedding *Salmonella*, we did not think it was fair to run her through a human machine that children would have to go through. The other problem was that you can not have any metal in an MRI, and you have to use completely plastic anesthesia machines which we really did not have access to. Here she is in the CT scanner, we are in the control room. Between shots we would go in and check on her status. I

would have to disagree with Dave [Jessup] about high tech rhinoceros, this one was pretty high tech. Initially we had a lot of difficulty trying to interpret the CT scans.

Bolin: Do you have any wild data on CT scans?

R. Kock: Give us one of those cones and we will!

Kenny: Scott [Citino] has done a CT scan on a rhinoceros also, so there has been two, but there are no normals. We had a human neuroradiologist take a look at it. It was actually quite good, but the difficulty was trying to comparing it to anything. I am going to read pretty much from here. There are two CT scans four months apart in this series. These are transverse sections through the rostral thalamus four months apart. He said there is a 15 mm hypodense area noted in the cerebral cortex. He is talking about this black area up here. This is the lateral ventricle. That he felt was a little more defined on the second CT scan. Here you can see the lateral ventricles and now they are larger, and here is the black hypodense area, he still felt it measured about 15 mm. There were also round hypodense areas present in the thalamus. This is a different level in the brain in the first CT scan, and there are symmetrical hyperdense areas now surrounded by a hypodense ring and are present in the caudate nucleus area. The hyperdense areas had regressed on the second study. You can see the lateral ventricles here and also these hypodense areas up top. On the first one, I did not see squat when I looked at it!

Anyway, as the animal's case progressed, what I did not really pick up on was that when you see the necropsy and the histological materials, is that a lot of the damage was present there probably at the initial attack at three weeks of age. What we were seeing was an animal that cleaned up its brain 15 months later. I was wondering if it was a progressive type of disease where she was getting worse and losing more brain tissue as time went along. Now I am starting to suspect that it was not that way at all. Most of the damage was there pretty early and how soon before we saw the attack, I do not know. Was she born with it and just handled that, and we could never tell the difference? I can tell you from first hand experience that a rhinoceros does not need a whole lot of brain to do a lot of the things that rhinoceros do, when you see the size of the lesions. Later on after she was stabilized, she was a little bit clumsy, and was *disphagic*, she never could eat on her own, and was small in size. But other than that she was relatively normal, and could do some clever things. She figured how to let herself out of the stall, even when it was locked. When we transferred her to another stall, she figured how to get back to that stall and get out. When the keepers lock me in the stall, I can not get out! She did some pretty clever things.

She went up to 15 months, and we saw at least 14 neurologic episodes that varied in severity to where see was blind, bumping into things, head pressing to sometimes just standing in the corner in a stupor. How many we did not pick up on, I do not know. After about 15 months her condition kind of acutely deteriorated. She also developed all of these skin ulcers, but I do not believe they are consistent with the other types of ulcers that have been reported in rhino.

In gross necropsy she had a multifocal dermal necrosis. Her cortex was bilaterally depressed with extensive cavitations. We will see her brain later. This is the first case from Denver, you can see some of these gray malacia areas here. This is "*Quanza*," it is hard to appreciate here but when her brain was extracted, both of these cerebral hemispheres were collapsed and compressed back by the occipital lobes. When they put her brain in formalin, it floated instead of sinking to the bottom. I knew there was something wrong then. We estimated that more than 50% of the brain's cerebral cortex had been destroyed. These cavities were filled with CSF fluid. The lateral ventricles they felt were enlarged, but not really particularly abnormal. The histopath... The lesions are very similar to the other three animals, the same regions of the brain basically, some focal mineralization. I think there was a little bit of a difference in the cellular reaction. Some of the acute ones had a lot more neutrophils, but this one was pretty cleaned up. The pathologist said it was really kind of boring from a histopath point of view, because there was just nothing there.

Etiologies... I just put down that poor vitamin E deficiency has been suggested. Certainly it has been seen in poultry as a cause of encephalomalacia. I have trouble with a toxin, how do you explain a toxin over a 12 year period, three different institutions, and only striking some young animals? Why did it not attack their mothers or other rhinos that were adjacent to them? So that one is a hard one for me to explain. Hypoxia... Originally when the human neuroradiologist looked at it he said, if this was a human he would suspect some kind of a massive hypoxic or *aschemic* event. So it is kind of a general category. Dr. de Lahunta recently in a letter to me, was concerned that he thinks it maybe it may be a genetic defect. The peripheral lesions he felt were the most interesting, because in those areas there were intact axons, cell bodies, the vasculature was intact. He felt that it was really indicative of a true leukodystrophy. There are some diseases in man that are comparable to this.

Munson: What were the PCV's of the mothers? Do you happen to know?

Kenny: I do not have any from the time in the beginning when she had the baby.

Munson: There was no evidence that the mothers were anemic at the time of late gestation or parturition?

Kenny: No, we did not immobilize them and take any samples, but there was no indication that there was anything wrong with them.

Munson: In terms of the hypoxic hypothesis..

Kenny: One event in this animal's life was that the mother was knocked into the moat by the male about seven months prior to parturition, [she was] on her back and struggled for about 15 minutes before she was able to right herself. Whether that could have had an effect on the fetus at that point in time we do not know.

Munson: But that did not happen with the other ones.

Kenny: No, that is not consistent then.

Miller: And partly in answer to that, the ones that were six months and two years at onset had been previously normal. They had not shown this stomach condition, enough for them to even retrospectively try and dig out a history.

Munson: But they could have had their own. I mean it may not have been maternal.

Kenny: I listed a few [human] examples [(metachromatic leukodystrophy, *globoid* cell leukodystrophy, adrenoleukodystrophy, Refsum's disease)]. I am not trying saying this is what it is. The first two are interesting. If you read the descriptions of the disease, they are really similar. Nice butterfly symmetrical lesions, and enlarged ventricles that expanded to the necrotic area that has disappeared. Again I would be interested to what Dan [?] has to say, because I guess it is again on this theme of enzyme deficiencies, but these diseases in man are autosomal recessive inheritive diseases. So this is the kind of scary concept. Could we have such a problem in our captive rhino's that would have profound effects on breeding programs? I really think of it as far as free-ranging animals, it has not been described, but obviously not every rhino gets necropsied either. These animals originally came from the wild, so if it is a genetic defect, it probably came in that way. It is something perhaps to be concerned about if we are going to be dealing with very small populations in the wild. There was another one that de Lahunta described that I have not been able to find yet, *Louise* disease in man. I guess human doctors like to name the diseases after themselves more than veterinarians do. Maybe we could call this Miller's *Myelin* Syndrome or something like that. These also have familial correlations, and certainly at the Denver Zoo you can construct a genealogy chart that will show some familial relationships. "Grindstone' and "*Leach*" produced "*Shakney*," which is the first animal we had with leukoencephalomalacia and they had other babies that had no problems; and produced the female "Onyx" that had no problems that we know of, but she is the mother of the next one that we had, "Quanza." Any questions

N. Kock: So far they have all been females?

Kenny: They have all been females. That reminds me of another thing. I karyotyped the next baby that they had, which was a male. When we had trouble with figuring out the karyotype, we sent it to San Diego. I talked to them and I asked them, have you ever seen an abnormal karyotype in a rhinoceros? They said just in one female that had a triple x sex chromosome. It turns out it is one of these females. The other three have not been karyotyped. I do not know if that is a coincidence. I would be surprised that you would see a gross genetic abnormality for a disease like this, but that is another little coincidence that seems to have piled up. Again, they are all females up to this point.

Blumer: Just to the field guys, certainly a calf like this would not survive in the wild, and then they would be hyena bait. Is there any anecdotal reports of neurologic calves circling or anything like that?

Kenny: It did not take long for this mother to want to reject the calf I think. She had given up on it real quickly after it had started these activities.

N. Kock: Can you karyotype formalized tissue?

Kenny: No, we tried that and they were not... They said it was remotely possible, and we had some on this particular animal, but no. One of the recommendations was that we should in future cases be sure that we freeze some brain next time for biochemical analysis.

Smith: If I remember right, the top two enzyme deficiencies were lysomal enzymes. I do not know about those particular two, but generally lysomal enzymes float out in the sera and you can diagnose alpha mannosidase or beta mannosidase, any of those. You can do frozen serum samples and get some idea. Because there is a normal level, and in infected animals, if it does not have any enzyme activity then there *is no need.*

Kenny: We certainly have plenty of serum.

Smith: I think what you have to do is find who it is that has got those assays up and running. Those particular two I think are not real easy. Some are real easy to do and some are real hard to do.

Munson: These are not typical lesions I believe of those two diseases you are talking about. I think what probably Sandi de Lahunta is implying is that there are genetic diseases that cause gross brain lesions, of which these are four examples. And maybe there is an entirely new one in the rhinoceros.

Sadler: I am a bit out of school here, because I am going to talk about some veterinarian medicine, but I brought this paper to our equine specialist, and his comment was that this is not uncommon in horses. In fact, the diagnosis and information that you have and the article is very similar. He said most recently they have determined that *gymnodicin* from *Fusarium* mycotoxins has been identified as a key cause of this problem in horses. Horses with apparently these same type of lesions. Apparently from South Africa they had the standards that allowed them to finally do the tracking that allowed them to prove it. I do not know if that is a track of interest, or...

Kenny: That is what de Lahunta was hottest on I think at first, but seems to have cooled off. How do you explain the three week old baby? Would it be getting it from the milk from its mother, it is not weaned. Where would she access it?

Sadler: Apparently it is shed. Apparently it does move through the system fairly readily. If it gets into the mother's system, it will move and be dumped into the milk. I think there is a case with *hortenel* horses that there have been some concern about.

Miller: We would be interested, because we had a hard time finding, we may not have found the right literature.

Kenny: Would it not affect other animals?

Sadler: The only thing I will comment there, and I am again really out of school here folks, but I did a study a number of years ago on polioencephalomalacia in cattle that was triggered by high sulfate levels in the rumen, causing a thymine deficiency. We did one trial where we had 76 calves that we thought should demonstrate polioencephalomalacia, and we could not pick one of them out. They all looked normal. We went ahead and slaughtered all cattle at the yards. We removed all brain tissues. We had 35%

incidence of polioencephalomalacia in those calves, and not one clinical sign showed up. So we had another dumb stupid steer that nobody knew was a little dumber than... I mean, your comments earlier, I do not want to make light of them, but you could easily from what we saw have higher brain lesions than her; and she just can not read the Sunday times anymore; but you probably would not know.

Kenny: The oldest one was kind of interesting too, in that it was moved from L.A. to Kansas City and it showed its signs about eight days after it got to Kansas City. Originally we thought it might be extrapyramidal effects of it gotten haloperidol. It was running into walls and traumatizing itself.

Sadler: But your symptoms against a polioencephalomalacia, I mean the head pressing, the blindness. We literally slapped things in front of these calves that did show clinical signs, that did not move or budge. And again to go back and find 30% some odd were effected with not one clinical sign, makes me think that you could have some other animals that have subnormal...

Kenny: I think there are other brains that are being harvested now because of this.

Miller: I think that was the point I was trying to make last night too, that this may at least for captivity, be a grossly underdiagnosed diseases. As Richard [Montali] said there is one at Denver that he is very suspicious of and *a lot* before that, being that these calves may have just gone down and the brains with the pathologist have not been processed or routinely harvested.

Sadler: I will comment that the main source of *Fusarium* and *gymnodicin* is going to be corn and wheat. Which is going to be a cereal grain and most wild types are probably not going to get the exposure if it is *gymnodicin* through that source. With the captive population on diets, probably you would have a better chance of seeing them.

Munson: The only thing is that I think you have to diagnose it, correct me if I am wrong, from the feed, and from isolating it. So the thing to take home from this is that you have a calf that is acting like that, right away isolate the feeding area.

Kenny: They analyzed the feeds for mycotoxins on the first animal from Denver, but the problem there is that that is not the same feed perhaps.

Munson: That is what I am saying, as soon as the clinical signs show up get the feed, freeze that.

Miller: Get that bag.

Kenny: The other animals were dead in about 24 to 48 hours, so it was pretty close to when it was first known that they were sick.

Sadler: Apparently these *gymnodicin* probes are just like within the last couple of years, according to him they have just finally got the standards *to do the* tracking.

Paglia: If you want to confuse the issue even more, at least throw in another variable looking for etiologies. We got interested in looking at glutathione peroxidase related lesions in humans that had neurologic problems, and there are some isolated... They are in the literature, whether there is a pure association with glutathione peroxidase and a certain kind of degenerative neurologic disease is still

controversial. Nonetheless, the association has been noted. I think we got interested in it because most of the black rhinos we had seen up to that point had abundant glutathione peroxidase activity, but *"Quanza"* did not. I think that is my recollection. More recently in going back through and trying to update materials for this group, I noticed that "*Quanza*" had the lowest catalase activity of any of the animals that we have measured. But there are a couple more things along with the other list of possible enzyme disorders that are related with neuro deficits.

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