HISTORY AND OVERVIEW OF THE PREDOMINANT DISEASES AFFECTING BLACK RHINOCEROS

Eric Miller

TAPE 1B 252

Miller: Before we get started I would like to add my thank you to all the supporters of this meeting including our hosts here at White Oak, and to Evan [Blumer] who conceived this and organized this much needed meeting. Evan [Blumer] first asked me to speak on historical perspectives of rhinoceros disease. Although publications in the scientific literature have been available from the Calcutta Zoo in 1893 and earlier, in reality it has only been about the last 20 years that more than case reports and series of articles appeared in the literature.

Although black rhinoceros contract tuberculosis and other routine diseases, what I will emphasis this evening is a series of rather unusual diseases that seems to affect this species with an alarming frequency. In the process I will try to present an overview of a cooperative veterinary research effort that started quite a while ago and is ongoing today. It maybe appropriate up front to stop and thank all the people that have cooperated in this effort. I think at least with the people I have contacted 270 and many people in this room and others that could not be here today, have really gone beyond *anything else with their* cooperative efforts.

I said it before in San Diego, but I still think that it holds, the diseases of the black rhinoceros are sometimes reminiscent of Winston Churchill's quote that they are "riddles wrapped in enigma shrouded in mystery." What I would like to do is go through these diseases a bit one by one. Many of them will be addressed in more detail tomorrow. Some of the ones like hemolytic anemia and fungal pneumonia I would like to give an overview on.

One of the best places to start on hemolytic anemia, for me at least, is the case that started my interest in 1981: a nine year old female black rhinoceros at the St. Louis Zoo, named "Martene." She was typical of the majority of what I call primary hemolytic anemia cases. She was presented with red urine which turned out to be hemoglobinuria. We anesthetized her for further evaluation and she had a packed cell volume of 14%. This was a thick blood smear from this individual. She died during the anesthesia, but not of an anesthetic complication, but a complication due to the *hemolytic* anemia. This is a sample of her urine, it could have been her serum. The hemoglobinuria and hemoglobinemia are quite remarkable in these cases. On necropsy we found a series of tissues with massive iron deposition. The pattern no doubt was due to the fact that there had been a massive spilling of iron. We will talk a little more about iron a little bit later and the work of Dr. Smith and of the Kocks in Zimbabwe.

The hematocrits on the animals on presentation in this primary hemolytic event range anywhere from 4.5 to 43%. The initial signs are hemoglobinuria, weakness, muscle fasciculation, and pale mucus

membranes if you can get that close. In these primary events generally death ensues in 24 to 48 hours. There are very few recoveries. We looked at a number of etiologies, equine infectious anemia, copper toxicity, and hemaparasitism. They all seemed unlikely in the cases in captivity. Lepto we will address in a few moments. Clostridial seemed unlikely. Vitamin E may be related. Hypophosphatemia may be related. Hemoglobin stability probably not. Autoimmune and metabolic will all be addressed in more detail.

We thought perhaps we had an individual case. When you are sitting out there in a zoo by yourself, you think *I have one of these strange cases*, we have seen a number of these diseases in other species. When we started to look around [we found] there had been previous publications. The preceding year, Mike Douglas at Memphis had reported cases at Memphis where three animals were infected and two died. He said they were suggestive of leptospirosis, based on elevated titers of 1:8000 to icterohemorrhagiae on the surviving individual. Thomas *Vagues* in England reported hemoglobinuria in a series of five cases in Great Britain prior to this also.

So we decided to undertake a questionnaire. It was mailed to all rhino holding institutions in northern America and western Europe. What we found was quite surprising to us. By piling data on year after year we found 44 episodes of hemolysis in 36 black rhinoceros, and 75% of those animals died during either their initial or subsequent episode of hemolysis.

When I say primary and secondary, the terms are not quite as clear cut as I would like them. But I call primary the cases that there seem to be no other underlying diseases, as in perhaps lepto. The animals develop hemolytic anemia and die. In secondary we are seeing a number of factors. I think they are all in a scale, I do not think they are on one side or another. There have been other cases of hemolytic anemia developing in animal's *parturnal* stages of other diseases, whether it be skin ulcers, or in one case there was a nasal abscess. Animals were stressed almost often medically for other reasons, then seem to develop hemolytic anemia in the agonal stages. I think that between there is a gray spectrum.

We looked at the data from a number of ways. There was no apparent sex predilection. It was a disease of adults in general. The range was two to 26 years, but the average age of onset was 10.7. Captive animals seemed to be affected slightly earlier than wild caught animals, but we are still not sure if this is statistically significant.

Interestingly enough, in many of the animals we saw no reticulocytes. We felt this was perhaps more like the horse not developing a peripheral reticulocytosis and *then go into an anemic crisis*. But we did see some nucleated red [blood cells] in a number of individuals. And indeed on the few cases where bone marrow biopsies were available, it appeared to be a regenerative anemia.

We mapped out family trees of affected rhinos. This is a family group at St. Louis. Four offspring are from a male named "Olive" and "Oil." Interestingly enough, the blue *cards* represent affected animals. "Olive" just died after stereo bouts of hemolytic anemia at Oklahoma City where she was recently transferred. So she is also an affected individual. Three of her four offspring died of hemolytic anemia. There were also family groups at Denver; and a grandmother, daughter, and granddaughter group at

Frankfort. What was interesting though, was that these three family groups seemed to be unrelated and they only accounted for about 12 of the 36 affected animals. There seems to be some relationship, but they do not account for the majority of the cases.

We know of some cases that were affected... There was at least one case at the National Zoo that developed hemolytic anemia while on Isoniazid therapy for tuberculosis. Interestingly enough, the side effect of Isoniazid in some red blood cell enzyme deficient people, what is most notably G-6-PD, can be hemolytic anemia. The Germans in Frankfort had noticed a possible response to corticosteriods. So then that made us wonder if there was an immune *implement* to this. In Memphis they had three animals affected and two deaths within a ten day period. Toronto had two animals die within a 24 hour period. However, in the majority of the cases there were other rhinos immediately adjacent exposed to feces, urine, and aerosols; and were not affected when one individual would die.

The previous reports by Douglas suggesting leptospirosis in his 386 case made us look at lepto right up front. Carol Bolin will address that in more detail tomorrow, but what became clear to us was about 50% of the cases of primary hemolysis were suspicious of infection of *leptospirosa interrogans.* Thanks to Carol [Bolin] more recent cases, where some antibodies have been applied, have been more definitive. The others it was *based on entire evidence, for instance silver stain for spirochete*, but a little less definitive in the availability of the FA. However, I think it is important to note the FA showed that not all of the cases of heterohemolysis are due to leptospirosis.

At the current time our recommendations are, kind of if you will, shotgun about how to treat hemolytic anemia. If someone calls me on the phone I will say use something like penicillin or aminoglycoside up front as if it was leptospirosis in the acute form. On phosphorus supplementation, we need these animals in the more chronic stages of hemolysis, not usually in the acute, but sometimes have had hypophosphatemia. Now, I think even if they have normal phosphorus, after talking to Don 408 at some length, there is evidence that phosphorus supplementation even in normal phosphic animals apparently helps the red blood cell metabolism.

Vitamin E we do know does have a role in membrane stability, and E in these animals is low. It has generally been given by injection. In these cases the injectable Vitamin E has been very successful in raising blood levels. And then in the few cases we have been able to monitor 416 the transfusion.

When feeding plant species in zoos trying respond to the fact that these animals are browsers, we tell them to avoid plant species that would be... Certainly things that are obvious, not browse species but like kale, grape, or cabbage that are known to cause hemolytic anemia in domestic animals and onions. And things like red maple that have been shown to cause hemolytic anemia in the horse.

We thought that we maybe had some proximal causes for the hemolysis, but we needed to take a broader view. Was there a common denominator that might allow leptospirosis to be the component in these cases, and Isoniazid in that case? We started calling around and got together a number of researchers of rhinoceros 431. One of the original studies was with Dr. Hugh Chaplin from Washington University in

his immune studies. He mentioned they were apparently responsive to corticosteriods in Germany. So Hugh made us some very predictive little agents. We had our own anti-rhino IgG, and then he made us our own anti-rhino whole serum; so we could test specifically for the immunoglobulin that is associated with classic autoimmune hemolytic anemia. And then we could shotgun, if it was maybe perhaps one of the other immunoglobulins that was involved. In the five animals that we were able to test that were in the middle of hemolytic crisis, they were all negative for autoimmune disease. Hemoglobin instability has been suggested as a possible source of hemolysis in these animals. However, we were able to identify with Dr. *Vergil* Fairbanks of the Mayo Clinic, that the hemoglobin was indeed very stable in this species, and there was no indication that it was involved.

Ellen Dierenfeld undertook Vitamin E studies which she will address tomorrow on its role as an antioxidant and its role as a membrane stabilizer.

Phosphorus and calcium have been suggested, particularly by Dr. Don Gillespie at the Cincinnati Zoo. During the midst of these acute hemolytic crises phosphorus has been normal; but it is a little difficult to evaluate, because obviously lysing cells spill into cellular phosphorus. I think what to me is more intriguing when I went back and looked at the data after being challenged by Don Gillespie and Don Paglia, was the ones that had the most marked hypophosphatemic episodes have often been the ones that have had the most chronic disease--long drawn out contracted disease with skin ulcers, or hemolytic anemia that came and went. It is almost as if with the chronic disease they are unable to mobilize the phosphorous out of their bones. I am sure we will talk a little more about that later.

One of the thoughts that occurred to me as I was going through medical texts in exasperation, was I read about G-6-PD deficiency in people. It was interesting to me that it was diagnosed in the Korean War when many of the American *black GI's* were put on antimalarials and a certain percentage of them developed hemolytic anemia. In back tracing, they figured that it was this enzyme defect. It was interesting, because it was a disease that could have a number of triggers and a *dull onset*. I called Don [Paglia] at UCLA, and I think it is fair to say he thought we had an easy fix too perhaps. It looked pretty clean cut. That started perhaps one of most intriguing sets of research projects that has been ongoing. Don [Paglia] looked a number of metabolites, which he will address on Sunday morning in detail, of the energy cycle of the red blood cell of the black rhinoceros.

I think it could all be summarized in Don's [Paglia] words, they are metabolically a bit *novel*; and working in a way that they predispose them to hemolysis and other diseases. There have been some spin-offs from this research that maybe do not directly address why the hemolysis occurs, but we are trying to develop a data base that will help us address it and other issues in the long run. Dr. Steven *Stockton, who could not be* with us, at the University of Missouri is doing all hematologies and serum chemistries in his lab so we have one central lab in the US that we can do our comparative data at.

I mentioned that we wanted to transfuse them. We have transfused one infant in the middle of disseminated intravascular coagulation. We also have transfused the animal that I had mentioned that had

the chronic nasal abscess that lead to hemolysis. Using 12 liters of blood from two animals we were able to raise the packed cell volume from 12 to 18%. However, the animal continued to hemolyze and die. We are unable to say if that was just a continuing hemolytic process in that animal, or if indeed it had a transfusion reaction. So we initiated a study with Dr. Ann Bowling at the University of California at Davis. It appears that rhinos are like dogs, they may have some blood groups like horses. The initial transfusion can be done. She has now typed the 523 serum 523 animals and she crossmatches that with about twelve series *of blood cells that have come in from 12 animals.* It appears that the initial transfusion could be done with out a great deal of concern. However, subsequent transfusions may put them at some risk..

Dr. Smith at Kansas State University noticed these high iron levels in our animal and others in the midst of the hemolytic crisis. There have also been some casual comments in the literature that iron hemosiderosis was seen in these individuals. The patterns that we could see were not that of an iron storage disease, but perhaps of a more chronic hemolytic event of hemosiderosis. This is the liver with centrilobular necrosis and the iron deposits from our individual that died at St. Louis. More recently, the Kocks from Zimbabwe were able to show in a very nice study that the newly captured animals, I believe the numbers were about a half a dozen, did not seem to have these iron deposits. Animals in short and longer times in the bomas in Zimbabwe have certainly been compared to the captive animals. Iron deposits seemed to be to a certain extent a function of time in captivity.

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Another spin-off of this was all these projects took quite a lot of blood. At one point we figured that if you completely fulfilled the Rhino Protocol we distributed to all rhino holding institutions in North America, it took 500 mls of blood.

We also emphasized comparative studies. We asked for samples from white and greater Asian one-horned rhinos for comparison. We have not gotten very many samples from those groups, but it certainly has presented some interesting data for the comparative study.

I mentioned that we needed 500 mls of blood, and so what behooved was to find another place to bleed rhinos. The ear vein is being used on this calf. We impressed on people to try and use the radial leg vein that really is quite large. We have been able to bleed in these blood collection studies and in these transfusion studies up to ten liters out of that vein in less than 15 minutes.

Another thing that came out of this was the establishment of central storage banks in North America. In St. Louis now, for the black rhinoceros, we have approximately 400 serum samples from 25 individuals. This has been a great aid in research. If Don [Paglia] or Carol [Bolin] or anyone else in the room calls up and says we would like to do a project with this, if it is done on serum we can produce the samples and have them mailed out within a week.

It has lead to formalized tissue banks with the black rhinos with Dr. Montali. Also frozen tissues, I can not emphasize enough the importance of this frozen tissue. When the FA became available with Carol Bolin we were able to pull out retrospective tissues. When we have been looking at toxicological studies

with Dr. *Brazin*, we were able to go back and pull out those tissues. So in addition to the serum, we have also ultralow frozen tissue in this bank.

It lead to the establishment of annual veterinary updates and reports. Because what became clear for a number of species, not just rhinos, was that it is critical to have someone in a central position in the SSP. We will talk about fungal pneumonia in a minute. It became clear it was a syndrome only after I had seen three or four of them. It was not a rocket scientist *identifying it. It was like gosh, it is three or four diseases, it is kind of unusual.* For many of these species it is very critical to have someone seeing the data all together in one spot. We produced a veterinary bibliography for rhinoceros to try and pull the literature together in one spot.

Let us go on to a few of the other diseases. I will not go through mucocutaneous ulcer disease in great detail, because that will be done by Linda [Munson] tomorrow. But this has been a disease that rivals hemolytic anemia in its prevalence. I have not talked to her recently about the most recent numbers, but in 1991 at the San Diego meeting she was able to report on 26 cases, and now I believe those numbers are in the mid 30's. These are all animals in the US zoos. It can present as oral ulcers, or in some cases massive almost sloughing of the skin, almost reminiscent of the horror pictures in vet school of epidermal 042. Linda will address that in more detail, but has been able to find no definitive diagnosis for these animals. The reason I mentioned the US, is that this is not the filarial dermatitus that is seen in Africa. We would certainly look for fliarias right away. Evan [Blumer] said we are looking for a common thread, and I think we can even expand that into a common web of disease. Many of these animals go on and develop hemolytic anemia.

Fungal pneumonia in black rhinoceros... We know of six cases in the United States and there may be more in Europe. On culture of the six animals, there were two with *Phycomycetes* and five with *Aspergillus*, one had two. The interesting part was that there has been four males and two females, probably just by chance. Four had been immunosuppressed, animals that were on corticosteroid therapy for other diseases, and two were spontaneous. No corticosteroid therapy, but one of them at least had the skin ulcers, and you wonder if they were immunosuppressed from the disease.

Evan [Blumer] had undertaken a year ago with another researcher to look at the sensitivities of these animals to corticosteriods. I can not emphasize enough to exercise caution using corticosteriods in this species. Many of these animals with fungal pneumonia have been on less than a weeks worth of therapy with 0.5 to 1.0 mg/lb of prednisolone; doses that according to the equine clinicians I have talked to would not put a horse at risk for this sort of disease.

Blumer: Dr. John Turner at the vet college of Ohio in looking at cortisol levels in rhinos, at least in the white rhino right now... He does not have some final numbers yet on the black rhinos that we have submitted. They are finding resting cortisol levels that are an order of magnitude lower than any other mammals they have ever looked at. Now, in talking with Mike [Kock] that is a bit in conflict with some of

the data that they found from the free-ranging animals. Whether that has to do with the fact that those animals have now been captured and cortisol levels go up as a result from stress of capture, I am not sure. But one of the scenarios that they think may possibly explain this is that, and it is a bit of a stretch, but we are here to stretch a bit, if these animals are used to such low levels of glucocorticoids and when they get exposed to higher levels, whether from stress or from exogenous sources, the multisystemic deleterious effects of those glucocorticoids may 072 themselves much more readily. That is all I can say about that right now.

Miller: We do not have any dosage information yet. One of our recommendations had been... Cincinnati recently had an animal in agonal ulcer disease. The ulcers do seem to be responsive to steroids. The stories are antidotal, but they are so responsive that the fact is that something is going on there. So I have said use them. I mean, the animal is going to die, but then they have also put those animals at the same time on ketoconazole or some other antifungal.

Jessup: Wouldn't that depend largely on whether you use very short acting or very long acting corticosteriods? It seems like even if they have a lower basal cortisol level, if you use a relatively short acting product, you should not see a prolonged effect.

Miller: It may be that we are going to have to choose another product. Most of these... One occurred on dexamethazone, which as I said is not as short acting. The others have all been on prednisolone. And obviously there is a variation in the various forms of prednisolone. But, none of these have been on *suppository* corticosteroids.

Harvey: Have you looked at the lymphocyte count on 084 steroids?

Miller: No, we have not, except on the Cincinnati animal. I would have to go back and look that data up. On most of the others it was not available. They died without blood sampling work. There was not blood sampling done while they were developing the fungal pneumonia.

Harvey: Because even with in carnivores, cats are fairly resistant and dogs are fairly susceptible and so to extrapolate to horse... It would be nice just to be able to see if you get 088.

Miller: This also baits the question about immunology. We have a proposed project subject to funding with Dr. *Slav* at St. Louis University, who would like to look at especially *Aspergillus* 091 diagnostic tests, but also some lymphocyte function tests.

The last of the big four diseases is encephalomalacia. This particular syndrome is effective in only four individuals. But it is one of the most dramatic syndromes that we have seen. This is the brain of the case from St. Louis. This is all necrosis over here, and this is the limited normal brain that is left. I think it may be a grossly underdiagnosed disease. This animal was presented being morbid and died. If we could not have sampled the brain, we would not have been able to diagnose the case. I wonder, [because] most people were not salvaging rhino brains in the past, how may of these cases have not been diagnosed? They do not always show blatant overt neurological signs. What was most impressive to me was that we

reviewed these cases and published our article with Dr. *Phil* 102 at Cornell, who is probably one of the top veterinary neurologists in the country; and he said without a doubt, these were the most significant far-reaching malacias in these three individuals, and now the fourth has come up, that he has ever seen in his veterinary career.

The pattern is primarily of leukoencephalomalacia, there is some polioencephalomalacia, but he felt strongly that the gray matter was only affected where adjacent to the white matter and it was an extension of the white matter lesion. We looked at a variety of differentials, and none of them seemed very likely--bacterial, viral. For years people have suggested vaccinating rhinos for equine encephalitides, but not only were the cultures negative, there was no histological evidence of a viral infection. Toxic, clostridial, 115 toxicosis and so on. The closest thing that he could come to was the pattern of leukoencephalomalacia seen in moldy corn poisoning in horses. I do not know if that is what it was. Actually, maybe we can discuss that more tomorrow during Dave's [Kenny] talk, because 118 *Bill Sadler* pass along some interesting information. These animals present anywhere from... Three of the cases were depressed, to one that was hyperexcitable. I think this probably reflects 120.

The other interesting series of diseases has been hepatic disease, ranging from liver failure noted by a number of people. Dick [Montali] will address this in more detail tomorrow. There have been bile duct proliferations, there have been some hepatocellular carcinomas. The iron storage we mentioned, but it really does not appear as iron storage disease, but more like perhaps the iron overload syndrome or iron necrotic hemolysis. More recently, the deaths of recently imported rhinoceros and several in the US have now been identified. Is it four you know of now Dick in the US that have had the same syndrome? It was with hepatic necrosis and bile stasis. The etiology appears to be toxic, and we will discuss that more tomorrow.

That was a bit of a whirlwind tour, but in summary I think it is fair to say that hemolytic anemia has served as at least as one trigger for a broader view of rhinoceros disease and functions. Vital to these studies of these unusual diseases of black rhinos has been concurcive with other rhino species. In one case, that of a white rhinoceros, pointing out their apparent health. When you look at white rhino health records, they die of a number of diseases, but there does not seem to be the pattern of these unusual diseases that you see in blacks. And perhaps, although it is still too early to tell, in the Sumatran [rhino] there seems to be a growing list of similarities with the black rhino. The search for a common denominator for hemolytic anemia has been broaden into a search for a common denominator of these diseases.

Metabolism is one promising area of study. And to paraphrase Don [Paglia] as he said, metabolically *novel*. In reality, these as I said may not be threads, but a web of diseases together. One of the most interesting conversations I had with Don [Paglia] was when he said, what would you say if I thought that some of these enzyme defects I am finding could link to more than one disease? Some of them have been perhaps associated with skin ulcers, some may be with encephalomalacia. It is not a stretch

to begin to wonder. And so we would have the grand unifying theory of rhino disease, and it would be the grand unifying theory of the universe as far as we are concerned.

Nutrition is another area warding investigation. At the 1986 rhino meeting in Cincinnati... I will try and paraphrase Richard Kock. I hope I do not do you injustice here. He put it in the best nutshell that I have heard so far. White rhinos are grazers and we feed them hay and they do OK, black rhinos are browsers and we feed them hay and they do not.

I think we meet such as this to also offer us an opportunity for an additional method of comparison, that of the management of rhinoceros in both the wild and captive communities. It seems to me that as many wild rhinoceros are being protected in relatively small often fenced sanctuaries, and as the captive community seeks the growth at cooperative national and international breeding programs and the use of larger facilities, our management skills between the two communities can only grow more similar with more to share.

Hopefully, a legacy of this meeting will be an approach to rhinoceros research based on identified needs and shared interest. As a result, perhaps instead of rhinos being killed for medicine, we can apply our medical knowledge for their benefit. In a small way, perhaps our study will help prevent diseases that have limited the growth of the captive population and complicated the movements and translocations in the wild, with hope for the preservation of the whole species. I used to say that black rhinos may not be around for our grandchildren, then our children, and now it seems even for ourselves. On that note, it may be applicable to end with a commonly overused quote, but I think it applies. That, "although a loss of a work of art is a tragedy, we can hope that some future artist will be reinspired; but when the last individual of a race of living things breaths no more, another heaven and another earth must come to past before such one can be again."

That is kind of a whirlwind tour through rhino diseases. I think it is important that during the course of this meeting that we look at things individually, but that we also try to be open; because I think they are certainly clinically connected, and I think maybe metabolically, nutritionally and all the other ways connected.

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