LEPTOSPIROSIS IN BLACK RHINOCEROS

Carol Bolin and David Jessup

DISEASE SPECIFICS AND LABORATORY ANALYSIS

TAPE 7A 054

Bolin: ...just in the past year. Tremendous geographic variation 055 both within the United States, different regions of the United States, and certainly different parts of the world. We have very little information about all the different serovars that occur in Africa. We are starting to get some of that here, there is some good lepto research going on in Africa and in Zimbabwe as well. So when we talk about leptospirosis in captive rhinos in the United States, I think we need to keep into that and not generalize to situations throughout the world; although I do have some information on Australia which we will talk about as we go through here.

Epidemiology--once again I just have boring cows, not rhinos. The United States Department of Agriculture does not know I am working on rhinos. They are not convinced that is an agriculturally important species. These are the common serovars of lepto in the United States. I would say the same is true in Australia, the primary serovars are *pomona* and *hardjo*. In Africa 065 some of this information, but we know that serovar *hardjo* is primarily the one that is in cattle. I do not have a lot of information. Also *tarassovi* is involved in parts of Africa as well, it does not occur in the United States. As far as imported captive rhinos, these are the ones we need to worry about in the United States, they are also the ones that are in the vaccine. Which is an amazingly coincidence.

The important thing about leptospirosis, and this is for all species, is that each serovar has a maintenance host, one or more maintenance hosts within the geographic region where it is established. These are the maintenance hosts for the United States and I will tell you where there are differences when I know them. *Hardjo* is by far and away the most common cattle lepto throughout the world, it certainly is in the United States. Its principle maintenance host in the wild is cattle. *Pomona*, another one that is quite significant in the United States is host maintained in pigs, cattle and skunks. *Grippotyphosa* [is host maintained] in raccoons primarily, although opossums in some parts of the United States. *Icterohaemorrhagiae*, which is one of those that has been indicated in some of the rhino cases of leptospirosis, [is host maintained in] rats, rats, rats, and nothing but rats. *Canicola* [is host maintained in] dogs, some of the wild canids can also be infected with *canicola*. *Bratislava* [is host maintained] primarily in pigs in the United States, maybe horses; and there is also significant *bratislava* titers in some wild caught rhinos, which Dave [Jessup] will talk about. Horses are a maintenance host in some parts of the world, perhaps rhinos are, for *bratislava*. I would doubt it, but you can not tell. House mice have been shown and field mice have been shown to carry it. I am not sure how epidemiologically important that is. *Ballum* is another serovar that is pretty rare in the United States, but is also found in mice. So I would think in zoos

your primary problem would be raccoons and skunks getting into your areas, rats are always a problem wherever you have feed, and mice. I would think that most of the captive rhinos would not have much contact with pigs, or at least I would hope that would be the case.

The important distinction here is that lepto is two different diseases based on which host it is in. In the maintenance host it is usually subclinical, a very high incidence of infection. Normally it infects young animals and pregnant animals. It is largely a reproductive disease. The shedding in the urine of this organism is long-term, probably life long in the maintenance host. Very low titers of antibodies develop in those maintenance hosts. It is extremely difficult to diagnose.

However, I think in the case of the rhino and most zoo species, there can be incidental hosts--epidemiologically dead end hosts. It is very rare for the infection to be pasted from one incidental host to another. And that is part of the reason why when we have collections of animals, you may see one animal with lepto, and the one that has been in the pen with that animal is not infected. It is not transmitted from incidental host to incidental host. And thus the incidence of infection tends to be quite low. However, it can be an acute severe devastating disease. Certainly human infections can be so, and in all species of animals including rhinos. It can infect all ages, although the mortality tends to be higher in very young animals. That may not be true in rhinos, but in general. The shedding in the urine is quite short term. In some species it does not occur at all. So urine is not always the best specimen to look in in these infections. Extremely high titers of antibodies are produced, and I think rhinos would fit into that category. Horses are another one that produce extremely high titers of antibodies, such that antibody tests are pretty reliable. The diagnosis is relatively easy. In other words, there are large numbers of organisms in the tissues if the animal dies, and because of the high titers of antibody it is pretty easy to find. So I think in comparison, lepto in rhinos certainly fits into this category, where we can make the diagnosis I think reliably, and we can exclude the diagnosis I think reliably. So in a way that is a big advantage.

Just a very little bit about the pathogenesis. Routes of infection--any mucus membrane will do. This is certainly true for our domestic species, and we have every reason to believe it is true for rhinos as well. It doe not take a bite from these wild animals, the urine of the wild animals is contaminated. Splashes or contact of that urine, whether it be ocular, oral or reproductive tract mucosa... The reproductive tract is primarily an issue in maintenance host infections in which venereal transmission occurs and is quite common. Skin, intact skin, particularly rhino skin which is an inch thick, I think is a pretty good barrier against direct penetration; but certainly through any wounds in the skin, or maybe even *palarial/pleural* lesions. That might be a good way to get the leptospiras through. So any mucus membrane.

What happens after they penetrate a mucus membrane? The organisms replicate somewhere, we are not sure where. They can first be detected in the blood four to six or eight days after the initial infection usually. They replicate quite readily in the liver, spleen and central nervous system, and kidneys. It is at this point when acute leptospirosis occurs. In cases in which the animals is going to survive, circulating antibodies develop. The spirochetes are cleared from the *parantenence* organs, and from the blood with the

development of these antibodies. Then the organisms persist in what we call privileged sites, which is primarily a kidney. That is how the carrier state and shedder state is set up.

Now I think in the rhino, what is happening is, animals are getting in this stage and dying. And because of this type of pathogenesis, if you are depending on an antibody test to make your diagnosis, the animals may die before they develop antibodies. So it is in this area, particularly in rhinos, where I think looking with a couple of techniques, florescent antibody test, etc., can make the diagnosis before those antibodies develop.

There are a variety of clinical signs in a variety of species. But I think this pretty well sums it up. You can see almost anything, but fever can be quite high, 41 or 42 degrees centigrade has even been reported; anorexia, obviously going along with that fever; hemolytic anemia and hemoglobinuria occur in some species with some serovars. I would say clearly it occurs in black rhinos with serovar *icterohaemorrhagiae* and probably others. But it is not always predictable.

Miller: It may be interesting to say that the ones in Czechoslovakia had high titers of *grippotyphosa*. That is why we only had a serovar, we 143 too.

Bolin: Most sero spiras and leptospiras do have the genetic material to produce hemolysins. However, some of them do not appear to do it *in vivo*. Other strains like *icterohaemorrhagiae* or *grippotyphosa*, or *pomona*, consistently produce hemolysin *in vivo*. The red blood cells of different animals have different levels of susceptibility to these hemolysins. Don [Paglia] and I have talked about this once, we have purified hemolysin, let's get some rhino red blood cells and put those two together and see what happens. But we have not had a chance to do that yet. But, needless to say, there is a potent hemolysin 151 produced by leptospira which I think is 151. Jaundice soon ensues as a result of these. Uremia and renal failure can also occur. Death is possible and appears to be likely in rhinos. *Abortion* 154 are not likely to be a problem in rhinos, more of a cattle *slaughter*.

The important thing if you suspect leptospirosis, which I would think we would have to in all the cases of hemolytic anemia, is to collect all the right samples. I think that is part of what we are here for. Serology is important. Acute and convalescent serum are very useful. A single serum sample ten days or so after the onset of the disease is also helpful. So even though we do not always have acute and convalescent, the titers that I have seen in animals that have survived and been documented to have leptospirosis are pretty high. So there is very little problem. If you had an animal that dies in that 12 to 24 hours or 48 hours after they develop the hemolytic anemia, chances are the serology, even in lepto infected animals, is going to be negative.

Dark field microscopy can be used, fluorescent antibody tests can be used to find the organism. Histopathology can be used to find the organism, but it is somewhat sketchy. You can use culture. We have all kinds of new DNA probes and PCR, but they are not really commercially or widely available yet. So I will talk about each of these techniques just very briefly.

Serology is widely available. It is relatively specific. There is a lot of cross reaction. The antibodies against one serovar will cross react with another. So what you tend to get back is a very high titer, let's say 8,000 to *pomona* and there will be titers to lots of other serovars as well, but they will tend to be lower. Vaccination, certainly many many rhinos in the United States now are vaccinated. That does interfere. They produce very nice titers as a result of vaccination. And so therefore, we are now going to be in some what of a compromise position to use serology as a tool to detect leptospirosis. I think that underscores the need to use other techniques. So whenever you submit samples, a vaccination history is really vital. Everybody has been really good about submitting those. Serology is insensitive for some serovars. That tends to be in maintenance host infections. It does not appear to be the case in rhinos. The serology is very difficult to standardize. We have been running I think most of the rhino samples. But if you split those samples and sent some to our lab and some to any other lab in the United States, I guarantee you, you would get different results. As to who is right or wrong, we are a national reference laboratory, so I hope we are right. But, the message is to use the same lab and use it consistently. I think that is true for a lot of the things we are talking about.

Serum antibody can be measured by the agglutination test, which is what is usually used. We also have ELISA tests, but we do not have them standardized for rhinos. The microscopic agglutination test is influenced by a number of things. First and foremost, specimen quality. Hemolysis does not bother us at all. We can read through hemolysis, that is the good thing. But, if it is contaminated, this is a live antigen we are using, and if it is contaminated with other bacteria or if there is a lot of fibrin in the sample, that is not good. We do use live antigens, and they tend to poop out after a while, so we need to refresh those antigens. Again, lab to lab variation is incredible. And it is a subjective evaluation, so that a two fold change in titer on two different days means nothing. Four fold, I start to pay attention, because that is probably not just day to day variation of the test.

Dark field microscopy is a very quick field test which can be done. Some of the rhinos have enough organisms in their tissues that I think if you did a wet mount of a liver impression or something, you could see them. You have to have a very good dark field microscope. You have got to have a lot of organisms. Unfortunately the false positive rating is very high, because there are strands of fibrin. Cilium will wash off the respiratory tract or out of the urinary tract or reproductive tract, and the cilia look for all the world like leptospiras when you look under a dark field microscope. So you need to be a little bit careful. But this is something that can be used in the field.

FA [fluorescent antibody], this is what we are running and had most of the success with since my *tenure at the DC* on the rhino samples. Fluorescence 202 is not specific in that it can not tell you what serovars involved, but it is specific for leptospiras. It is not good to detect for *Borrelia* or *Treponema* or other kinds of spirochetes. It sensitivity, while not as good as culture, is really pretty good. I do not have any problem in these incidental host infections, in which there is quite a number of organisms. The antigens have to be intact. I will show you an example of how often times the rhino samples that we get have been

frozen already. Sometimes they have been frozen and thawed several times. The animals were quite ill, sometimes sit for a while before their postmortem is conducted, and that tends to degrade the organisms. I will show you what that does to a sample in a minute. But you do not have to have live organisms, frozen samples will work. It is quick and inexpensive. In the important cases, we can have turn around times the same day we get the samples. We have new serovars specific fluorescent antibody tests which we are getting on line. So we will not only be able to tell you, that yes it is leptospirosis and depend on serology to sort out which kind; but we will be able to tell you based on FA that it is *grippotyphosa* or that it is *ictero*. And that can help sometimes in tracking down the source of your infection. But that is still a research lab tool at this point

SLIDES. This is a typical FA, this happens to be a raccoon, and this is what we see, this bright green apple florescence. That is the leptospira, there is no question about it. To call something positive, we have to see an intact leptospira. Just bits and pieces of fluorescence we will not call positive. The next slide I have is from one of the rhino tissues that was positive. Here you can see this kind of "stuff." The conjugate is really quite specific. So from all of my experience in domestic animals, this is probably bits and pieces of leptospiras, that are either inside of cells or have broken up in the freezing and thawing process. But we would not call that positive until we saw this organism right here. So that tends to be a problem. And if you have diagnostic labs that are not used to looking at this type of sample, sometimes you have to look for a long time to find that one organism. So the FA has really helped us and I think it works quite well in the rhino tissues, even with this tissue which had been pretty well beat up by the time we got it.

Sadler: Will you make a positive with just one?

Bolin: Yes, the conjugate is wonderful. If it looks like a leptospira and stains like a leptospira, it is. I do not have any problem with that.

Histopathology is often the only technique we have, because the only tissue that is available is fixed. However, you need to use silver stains and they are expensive and they are tricky. I just picked a couple of samples from my file. This happens to be a bovine kidney, and these are renal tubules. Within the tubules you see all these darker staining objects. Those are leptospiras, they do pick up the silver stain. The thread like organisms are leptospiras. You can tell they are not *Borrelia*, that is an easy diagnosis to make. They look very different in tissues. People are very worried about that in the United States now with lyme disease around. This is a tremendous number of organisms. We very rarely see that number. This happened to have been in an aborted fetus. So while it is good, often you will search four or five sections and find one organism.

Culture is wonderful if we get the tissues in the right shape and the right time, we can culture the organism. All those that occur in rhinos, as far as we know, are quite easy to culture. It is expensive and it is labor intensive. It can take up to 24 weeks to get a positive culture, which usually it is all over but the

shouting at that point. And it is extremely difficult and expensive. So we essentially have not been trying. Occasionally we can isolate something from frozen tissue. Because the FA has been working so well, we have not even been trying culture in rhinos. But if anybody had a suspect case that they really wanted to work up, call us in advance so we would be set up to take it and put it quickly in 255.

Human infections, I will get up on my soap box for a minute. All mammals are susceptible to leptospirosis. Cats are questionable. But all mammals including human beings are very susceptible to leptospirosis. The most common time that humans are exposed is when they are assisting at calving or milking the animals. But surely anytime, the types of intensive management you are doing with these ill rhinos, you are going to expose everyone who handles that animal. Just like in domestic animals, any mucus membrane will do. Often it is a flu like illness, but it can be very severe and fatal in human beings even with antibiotic treatment. Pregnant lactating women...in the swine industry we have a big problem with this because the people who work in farrowing houses often are young women of child bearing age. They do very well in the farrowing house, for whatever reason, which I think is beyond the scope. The organism passes the placenta in humans just like it does in any other species. It can be transmitted through the milk to offspring. Before we started vaccinating them, 50% of [the sick rhinos] were documented to have lepto. So those of you handling these sick rhinos --wear gloves.

I think the vaccine contains all of the serovars. I do not know if people have started using the six way vaccine containing *bratislava*, but it contains all the serovars which are common in the United States. The vaccine titers appear to be good. Once every six months seems like a very reasonable recommendation to me. If you are going to be injecting your rhino at five months or at seven or eight months, I probably would not capture the animal again to inject them in six months. The duration of immunity is likely to be pretty good, and I think it will solve most of the problems. I think that the group that was imported from Zimbabwe had all been vaccinated before they came over, and I think that was an excellent plan.

Blyde: How do you vaccinate a rhino?

Miller: We have just been doing it with a dart pistol.

Blyde: How do you get the darts out of them?

Miller: We put them in the neck, and them when they walk up to the bars we just pull them out. And one of the side effects we have seen, and I have not quantified this, is that there does seem to be a higher percentage, at least in our individuals and I have heard from others, of injection site abscesses. That has never for us presented a problem. They drain quite well, instead of becoming these tremendous abscesses.

Bolin: One thing to also be a little bit wary of I think... I know you have had one animal you vaccinated with lepto at St. Louis and went down, right?

Miller: No, that was Oklahoma City that gave us that story. It sat down as if it was having a reaction and later stood up on its own. But there was not strong 292 that made him anaphylactic 292.

Bolin: Right, the only thing we have with this frequent vaccination, there is a lot of bovine serum albumin [(BSA)] out in all lepto vaccines. You want to be a little careful with that. If we use horses as a model, horses are not widely vaccinated for leptospirosis in the United States, although leptospirosis is quite common in horses in certain parts of the country, because of vaccine reactions. Now largely this is anecdotal. I have never seen one, I have never talked to anyone who has seen one. Everyone just talks about it. It is this anaphylaxis to the high content of BSA. The animals usually recover, very few deaths. But you can drop them sometimes when you are giving that. Someone has suggested, and again I am not sure how you would do this in a black rhino... The first dose is no problem, the second dose and subsequent doses, what they have started to do in some horses in Kentucky, is they will skin test the horse. They will do an intradermal injection. If the horse does not get a big wheal and flare, they go ahead and vaccinate the horse. If the horse gets the wheal and flare, they do not. Again, that does not seem highly practical in these rhinos. That is why I would not go any closer than once every six months.

Blyde: What are you doing Eric?

Miller: Every six months.

Blumer: Does it need to be that often?

Bolin: We do not know how long the titers last, and the protection lasts beyond the point where the titers disappear.

Miller: If it would be far to summarize, one of the things that we have lacked is the titer data that is in the paper, is drawn opportunistically. It is just a pooled set of data from individuals. And no single individual were we able to follow serially for the 314. The six month schedule was drawn from a number of recommendations. One partly from that pooled data, and partly from the recommendations of you and people in infected dairy cow herds, where they tend to go six months rather than annually.

Bolin: In California the dairy herds are all going every two to three months. But then you are putting bovine serum albumin into a bovine, you are not putting bovine serum albumin into a horse like creature, and that is my concern. Once every six months, I think lots of rhinos have been vaccinated at that rate. I would probably feel comfortable with nine months, twelve months.

Blumer: This is the kind of thing we need to be particularly cautious.

Miller: You are exactly right. The thing that we have talked about is if we can identify animals that are going into serial bleeding protocols, it would certainly be helpful to follow those individuals serially down their vaccination protocol, instead of trying to do this pooled data system.

Bolin: It takes 0.3 ml of serum.

Blyde: What are you doing Evan?

Blumer: We are doing every six months right now, but it is a little difficult because we have to do it by dart, and the animals are a little bit difficult to be darting. So we are doing it every three months anyhow to get a little shot. But there is going to be some opportunities for some studies and I am sure that

Mike [Kock] will be glad to talk more about this. The plans are at this point to put together an intensive management center in Zimbabwe where there is going to be a pool of animals that we can hopefully work on some of these projects with. So it is not just the animals that are here in the zoos or in Australia in the zoos.

Bolin: That would be good to know, because I think the less we can vaccinate them and keep them well protected, I think that is the best strategy.

Blumer: You drop one into major anaphylaxis, you are going to have a whole lot of people screaming, so...

Bolin: Yes, I here that, but how many rhinos have died from leptospirosis?

Blumer: But curators and directors are not thinking that way.

Bolin: I am working with a couple of companies now in developing a horse vaccine. The first thing we are going to do is take all that BSA out of it and cut back on the antigenic load. I think that when and if that... Of course the liability issue in horses makes the companies crazy. So whether we will ever get that on the market or not, I do not know. But that is what I would do. And if we started having problems, I would propose we make one that does not have any BSA. The technology is easy to make one without.

Blumer: Then lets make it.

Bolin: I would be happy to.

Miller: Could you comment... I think most of us were using the Norden product. How important is it that we stick with one manufacturer? I am not asking you to make an endorsement, but I have heard other clin-path people say that the Norden product is the most antigenic. Is there any truth to that?

Bolin: No, it is absolute malarkey. Almost all of the vaccines in the United States are formulated within a smidgen of the same number of organisms. Cooper's Biological had a product that had more *hardjo*, which was specifically the problem we were concerned about. But when you get more antigen you have probably got more BSA too. I think anybody's is fine, they are all the same.

Morkel: With lepto, is the main source of original infection in domestic animals through fecal contamination by rodents with *icterohaemorrhagiae*? Or is it housing, where is it?

Bolin: Any place there is urine. The organism can persist in watering troughs, ponds, the wallows that we give animals, for four months, if it does not freeze. It does not survive freezing and thawing and it does not survive *acid/ash in* soils. If you have mildly alkaline wet, and you have any sort of wildlife urinating in those premises, that is how it happens. But it is not 364.

Paglia: Almost any tissue can be infected, is that correct?

Bolin: Almost any tissue...when that septicemic phase, or the bacterin phase?

Paglia: Yes, do you see intracellular organisms in almost any tissue?

Bolin: You do not see intracellular organisms. The spirochetes are extremely delicate, they are killed very rapidly when they get intracellular. They are largely free. They will pass through, but they do

not hang out in there. But you can see them in virtually any organ, joints, eyes, the best place to get them out of the vitreous or aqueous humors or virtually anywhere. So you can see signs of anything, just like you can in people, aseptic meningitis, or see nothing, then they become lame during the youth phase of the disease. You can see virtually anything. But I think it would be very interesting to put rhino red cells together with purified hemolysin, and see what happens. In some species, nothing happens. It would be very interesting to see what happens with rhino.

PREVALENCE IN FREE-RANGING AND CAPTIVE BLACK RHINO

TAPE 7A 409

Jessup: This is obviously a collaborative work inspired and largely supported by Eric's [Miller] access to samples from captive animals and Mike [Kock] and Pete's [Morkel] access to free-ranging animals and Carol's [Bolin] willingness to run them. As I mentioned, this work on the free-ranging animals is an offshoot of the capture-relocation work being done in Zimbabwe and some similar work being done in Namibia. Without this kind of cooperation we simply would not be able to ask the types of questions in free-ranging animals that are such concern in zoo animals. My interest in this stemmed from reading the Cincinnati workshop and the questions about hemolytic anemia in rhinoceros and the speculation of leptospirosis as one of the prime causes of the hemolytic anemia syndrome. Eric [Miller] has already presented you with some of the information. The rest of it is readily available.

It does appear that leptospirosis has been involved in about 50% of the hemolytic anemia cases in captive rhinoceros, sometimes confirmed by rising titers, sometimes confirmed by FA, sometimes confirmed by silver staining, often not any one of those; but it does appear to be involved in this particular syndrome. I have had trouble, and I think all of us have had trouble up until just recently, separating the hemolytic anemia syndrome from perhaps some of the other icteric and haemorrhagic syndromes in rhino. Since they are so unrewarding to deal with and to treat, it seems like getting some basic information on the prevalence of leptospirosis and the efficacy of vaccination is well worth doing.

We were able to sample four areas in Zimbabwe and one area in Namibia. We were able to sample some of the animals captured along the Chenje River, some along the *Chewore/Kachowe* River, some at Mana Pools and some captured in the Makuti area. We also had some samples from scattered locations in the country, and some where the location was not relatively easy to... there were only one or two animals at a location, so I kind of lumped those all together as "other" when we look at the data. Obviously these areas, as you have seen from Mike's [Kock] slides, and Raoul [du Toit] slides and various other people's, vary in terms of the ecology and plant life. This is typical of the Zambezi Valley habitats that we were able to sample from. Along the Zambezi escarpment, animals [were] caught in that area, [where it was] higher and more well drained. And then we have seen pictures from Namibia, obviously ecologically very

you said Chewore River, but I thought you might mean Kachowe River, or is there a Chewore River?

different areas, different rodent populations, different small animals populations, different soils. The ecology and microhabitats in these areas may be quite important in the maintenance of leptospirosis.

Just to kind of summarize the data. We use 1:100 as the cut off, 63% of the animals sampled had titers of 1:100 to at least one serovar. Titers at least as high as 1:400 in 25% of the samples from the Zambezi Valley, just looking at the 1988 data. So it is a relatively common disease in rhino in Zimbabwe. But, interestingly enough, it appears that there are certain serovars which predominate in certain river drainages. For example, of 16 animals sampled in the *Chewore/Kachowe* River area, 15 of them had a 1:100 titer of *icterohaemorrhagiae*, there were a few other *tarassovi*, *grippotyphosa*. But it appears that *icterohaemorrhagiae* is the predominant serovar in that particular area. In the Mana Pools area it appears to be *tarassovi*, and along the Chenje River area it appears to be *tarassovi*. In the animals in the Makuti area along the Zambezi escarpment, very little evidence of previous exposure to leptospirosis. Namibian animals we were not able to, although 458 is pretty small here, we were not able to detect any previous or any antibodies to leptospirosis. It is not all unknown. These are mixed groups, one or two from one location, one or two from another. But the others that we threw in the unknown bag, again do not show any particular pattern. I guess this to me was somewhat interesting that there might be predominant serovars. Within particular areas animals may have relative resistance to one particular serovar, be moved out of the country, exposed to a different serovar in a zoo, perhaps one carried by a raccoon or a rat, and still be susceptible to clinical disease.

Kock: Just one point. I think that the main ecological variation in the Zambezi Valley situation will come through the difference of fire in these areas, not rainfall. In fact the rainfall is higher than in the valley 473 areas.

Jessup: Drainage I think is really probably... at least as far as the pools. If you look at the information from dairy ponds and alkaline pools in the United States, these types of pans and pools that are down in the valley are probably more capable of maintaining the organism in a place where it can be infectious to rhino, than better drained and less moist areas.

M. Kock: But in the Chenje and Kachowe areas 483 does not lend itself to 484 in fact there is a lot of 484 in those areas. Whereas in that Mana Pools area 485 that is where you have got this kind of situation. So there is variation in valley 486.

Jessup: Obviously it does not take a rocket scientist to see there is a difference between some of these areas in Zimbabwe, and some in Namibia. Some of the information from captive animals... It appears that vaccination, as Carol [Bolin] said, is reasonably effective. The various serovars are all in the vaccine. Probably every six months like in horses is adequate, but it seems like some of the things we have been discussing here, like whether it is really a good idea to be continuing to challenge these animals with this antigen is something we are going to have to look at more. Vaccination prior to moving animals to new

locations where they may come in contact with new serovars, like these relocated animals, or perhaps from zoo to zoo, is something that we ought to be considering.

Basically, that is about all I really wanted to say about this. There is a couple of other things I would like to mention. One is that although I was kidding a little bit yesterday about keeping Mike [Kock] and Pete [Morkel] and Rick [Kock] honest, really my interest in this and to some extent the role that I have evolved into is this sort of being their Godfather rather than their guardian. They have been able to get me interested in and involved in some of their work. It maybe possible for all of us to get a glimpse at what is going on in free-ranging animals. Our little organization primarily has been trading drugs and darts and equipment to them for serum samples that we try and make available to most of you. That is basically what IWVS [(International Wildlife Veterinary Services)] exists for, is to support our field veterinarians and to do as much as we can to facilitate finding out what is best both for captive and free-ranging animals.

But before I sit down, there is something I want to emphasize, basically it is something that Pete said earlier. A lot of the problems with rhino are not high tech problems. They are pretty low tech problems, or medium tech problems 531 chainsaws that medium technology. Although I am very supportive of what we are doing and what we are working for here, and obviously with the dismal events of the last couple of years, *in situ* conservation and *ex situ* conservation working together is critical. I hope that we do not lose sight of the forest for the trees. If all we save is captive black rhino, and we fail to do everything we can to support Raoul [du Toit] and Pete [Morkel] and Mike [Kock] and Nancy [Kock] and Rick [Kock], and people who are trying to do their best to save free-ranging black rhino, then it may be another heaven and another earth before we have these animals really again.

Morkel: Can I ask you a question about this fact that you have got such a high percentage of the Zimbabwe animals positive. Does that mean that when they are out there, they are obviously contracting an infection and resisting it well? Is that correct?

Jessup: Well, they are obviously coming in contact with the organism. Maybe Carol [Bolin] can answer this, but I would assume that they are becoming infected and obviously surviving, at least the animals that are sampled. The captive animal information would suggest that some animals develop acute clinical 556 and fatal leptospirosis. Whether that actually happens in wild animals or not, I do not really know.

Morkel: Might it be a dose related problem or might it be an immune related problem?

Bolin: Herd immunity develops, I know rhinos are not herd animals... If you took any domestic species, obviously I have not done this with rhinos, and lined them up and I gave the same dose of lepto to each of them, a fatal dose that would kill some of them, I would see disease in maybe half with the really virulent ones. Subclinical, particularly clinical leptospirosis like we see in the rhinos is a 569 not the most common. But when you combine it with stress and everything else that we are talking about, it could be

challenge dose, although that tends to change the lag phase, it does not tend to change the outcome. I do not know.

Jessup: Again, it is easy to speculate, but some of these "die-offs" that Raoul [du Toit] is talking about, you have to wonder whether if you have a number of animals urinating in a water hole and you have a very limited water hole, if you could lose some animals suffering from clinical disease and have a number of others survive with titers.

Bolin: That is the rule of leptospirosis in every species. There is far more titers than there is severe acute clinical disease.

N. Kock: I would think too that if you did have subclinical disease and hemolysis, then you would also have what I looked for in free-ranging animals, and that is hemosiderosis, which you do not have. Not to say you do not in another situation where there is other 590 but in a free-ranging situation.

Bolin: Yes, if it is causing the same syndrome that appears to set off in the United States with this massive hemolysis. But if it is just a little bit... In some animals the infection the antibodies may develop is fought off very rapidly, everything else is right with that animal.

Jessup: How long after a hemolytic crisis do you resolve... I mean how long does that iron 599 stay around? It is not going to stay around forever.

Smith: Forever.

Jessup: Forever? That answers that.

Smith: She had made an assumption that may not be true. There is just a limited amount of iron in the body. In order to get iron accumulated... If the PCV goes back to normal, the only way you are going to keep it in the liver is to increase the absorption. There are very few mechanisms to get iron out of the body. Well, hemochromatosis if we were talking about in humans. Once you get it in there, it does not come out very good at all.

Harvey: I have one question are these *seras still around*?

Jessup: Yes. Actually it is one thing that I guess I meant to mention when I showed that slide of the sera, we have got samples from 60 plus animals primarily from Zimbabwe. We have been offering them for use for diagnostic purposes to various researchers. Evan [Blumer] has had some interest in following some of them, although I will have to tell you what little I know about the length of time that Naphthalene is supposed to stay around in tissues and sera. Those certainly upon request can be made available.

M. Kock: I think you are right when you talk about research and things like that. This leptospirosis question has sort of expanded and some work can be done with Kenya and maybe some more with Namibia, and we have a lot more samples now from other areas in Zimbabwe that can be made available, just to try and expand the knowledge we have of this disease.

Jessup: One of the big things that came out of the Cincinnati meeting was a request for the availability for these types of samples. And certainly they have helped us answer or at least investigate a number of different health questions.

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