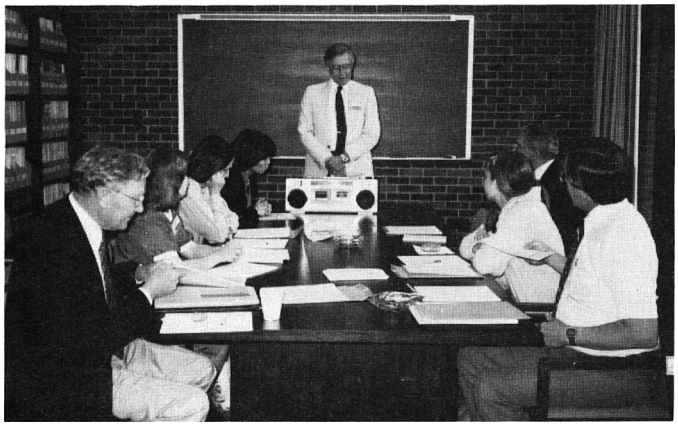
Conference on Malignant Catarrhal Fever

Oklahoma Animal Disease Diagnostic Laboratory
Oklahoma State University
Stillwater, Oklahoma
April 17, 1985

"One small step for man, one giant leap for mankind"
—Neil Armstrong – 1969, as he stepped on the moon.



Dr. Dan Goodwin, Director, OADDL, welcoming the conference participants.

Each journey into the unknown begins with one step, and the conference on malignant catarrhal fever (MCF) on April 17, 1985, at Oklahoma State University provided the first step in bringing together researchers on MCF from the United States and England. The discussion forum was led by Dr. Walter Plowright the eminent British virologist who was the first to isolate and identify the etiological agent of MCF from a blue wildebeest. The conferees gathered at the Oklahoma Animal Disease Diagnostic Laboratory (OADDL) at Stillwater, Oklahoma. Dr. Dan Goodwin, the director of the OADDL, opened the conference by welcoming the conferees and introducing Dr. Anthony E. Castro who served as the moderator.

Dr. Castro: We thank Dr. Walter Plowright for coming from Goring, Reading Berkshire in England to join us on this occasion. In January of 1984 in Arizona, Drs. Werner Heuschele, Ed Ramsay and I, along with Dr. John Mare had an initial conference on MCF which led to the present meeting. I will ask Dr. Plowright if he first will give us a brief historical perspective pertaining to MCF.

This report was prepared by Anthony E. Castro, D.V.M., Ph.D., University of California at Davis, California (formerly on the staff of OADDL) and Werner P. Heuschele, D.V.M., Ph.D., San Diego Zoo, San Diego, California.

Dr. Plowright: Research on MCF really began in South Africa in 1919 to 1920's. Melton described farmers who kept herds of cattle and black wildebeest grazing together which had cases of MCF. He also established that it was a transmissible disease by cattle to cattle inoculation of blood from an MCF-affected ox, but did not isolate virus. Early in the history of MCF, Piercy in Africa obtained a line of virus which was transmissible to cattle and also serially into rabbits. I later put forth the basic ideas of the pathology of the disease at the histopathological level as lymphoproliferative lesions in visceral organs and vascular lesions which suggested a similarity to infectious mononucleosis in man. We bled a number of wildebeest, and from cattle inoculations obtained 3 isolates of virus, one from a fetus which provided the clue that virus was partially maintained by *in utero* transfer. We also found the virus produced a cytopathic effect in bovine thyroid cell cultures. Thus in 1960, we were able to develop a strain of virus called WC-II after the number of the wildebeest calf from which it was derived. This isolate produced free virus titers of 10^4. Later, we used calf testes and got 10^6 titers of virus per gram of lymph node tissue from sick cows and 10^3 virus titers from blood. I also found that you could propagate virus directly in secondary calf kidney monolayers. Harkness and Jessett later demonstrated increased yields of virus by propagating virus at 33 or 34C rather than 37C. So what early appeared as anomalies of the virus disappeared with the passage of time.

Dr. Castro: Investigations on MCF in the United States have been limited, except at Plum Island. I believe Dr. Heuschele can comment on MCF at the San Diego Zoo (SDZ) because that is where it all probably began.

Dr. Heuschele: The first documented case of MCF at the zoo prior to 1974 was in a banteng, a wild bovine from Indonesia. We finally lost our entire collection of bantengs to MCF. Subsequently, between 1976 to 1980 we had at the San Diego Wild Animal Park (SDWAP) sporadic cases of MCF in Barasingha, Axis and Sika deer. In 1980, we lost 16 Pere David deer, an endangered species extinct in the wild, which was our entire collection. Lynn Griner while at the SDZ commented on the presence of lymphosarcomas in Sika deer at the SDWAP which later were shown to have vasculitis in the

tissues. Recently, we reported on the association of a lymphosarcoma in a Sika deer where recrudescence of MCF by dexamethasone occurred which likened MCF virus to the Epstein-Barr virus, a gamma herpesvirus, that in certain circumstances is oncogenic. In addition, this Sika deer was negative for bovine leukosis. Our first isolation of MCF at the SDWAP was in 1981 from a Nilgai fawn in fetal Pampas deer thyroid cells. Later we used fetal Aoudad (wild sheep) kidney cells for the isolation of MCF virus. From the Zoo nursery, we obtained MCF virus from 2 Sika deer housed with a baby wildebeest.

Dr. Crandell: Then are all cases of MCF associated with lambing or calving?

Dr. Plowright: Not in all instances! I remember an ox becoming infected in an area where 2 adult wildebeest were undergoing heat stress; however, the nature of stress in MCF is still in question.

Dr. Heuschele: We isolated virus (not cell-free) from a Sika deer in which we recrudesced MCF by using corticosteriods for 6 days at 0.1 mg/kg of body weight.

Dr. Plowright: If you take adult wildebeest under ordinary circumstances, they are not going to transmit MCF to cattle, unless they give birth. Then the MCF-affected calf will excrete virus which is not bound with antibody.

Dr. Whitenack: Dr. Heuschele, has it been established that several years ago there occurred transmission of MCF to cattle near the SDWAP?

Dr. Heuschele: The sum total of cows lost to MCF in the dairy adjacent to the SDWAP was 15 which was documented by Mare and Orsborn. John Mare also reproduced the disease by inoculating blood from the cows into calves and rabbits. This dairy cattle episode occurred in the winter, and the wildebeest herds at the SDWAP were calving in May, June, July and late August; however, the herds were in close proximity (in the South Africa and East Africa Plains) or 500 yards from a fence and road which was adjacent to the dairy. Mare did tell me he got some viral cytopathology in MDBK cells but he later lost the virus.

Dr. Plowright: The experimental incubation period for MCF is 3 weeks so you may be stretching this episode to the calving of wildebeest. Additionally, Mushi in Africa has said that IgA in the secretions comes into play in 3 to 4 weeks and neutralizes the virus.

Dr. Castro: Some of the questions raised in this discussion pertaining to transmission are: When is the cell-associated virus being shed? Is this infectious virus? How long does the virus survive outside the host? Do cattle to cattle contacts pose a problem? In 1979, an outbreak of MCF occurred at the Oklahoma City Zoo. Dr. Jensen, the zoo veterinarian, and Drs. Whitenack and Zimmer of the OADDL coordinated efforts to get tissues and blood from clinically ill animals. We were able to isolate from a gaur with clinical MCF an agent which produced syncytia after several passages in bovine fetal kidney cells. So we reproduced the disease by the inoculation of a heifer which died within 18 days with MCF, and the virus also killed 2 inoculated white-tailed deer. We were able to fulfill Koch's postulates. We then recommended to the zoo to cease breeding the wildebeest and cases of MCF at the zoo have dwindled in the last few years to nil.

How many viral isolates do you have, Dr. Heuschele?

Dr. Heuschele: Twelve of our own including 2 from topi and the WC-11 strain.

Dr. Castro: We have about 16 viral isolates in the U.S. but to respond to Dr. Plowright's previous question, "Are they all the same?" We don't know! Our biggest concern is, does MCF pose a threat to our domestic livestock and our wildlife (white-tailed deer)? There have been reports of a togavirus, a morbillivirus, and cytomegaloviruses as potential agents in MCF. Our evidence suggests that these agents are not involved with MCF. We are dealing with one type of virus and varying clinical manifestations of this MCF virus. We currently have a serum neutralization (SN) test for MCF to determine animal exposure to virus; however, the SN test is time consuming. We have developed an enzyme-linked immunosorbent assay (ELISA) which offers a rapid method to monitor animals for MCF.

Conference participants were, front row from left: Dr. Candice Metz, Ames, Iowa; Dr. Laurie Doyle, Emory University, Georgia; Dr. Siao-Kun Wan, OSU; and Mrs. Sandy Rodgers, OADDL Lab Manager. Back row: Dr. Dan Goodwin, OADDL director; Dr. Werner P. Heuschele, virologist, San Diego Zoo; Jack Kizer, Oklahoma City Zoo; Dr. Walter Plowright; Dr. Mike Worley, San Diego Zoo; Dr. Anthony Castro, OSU virologist; Dr. Lindsey Hutt-Fletcher, University of Florida; Ms. Jill Dotson, OSU Technologist; and Dr. Robert Crandell, head, microbiology, Texas Veterinary Medical Diagnostic Laboratory, Texas A & M University.

Dr. Heuschele: Dr. Plowright, what is the status of MCF in Africa today as it threatens the cattle industry there?

Dr. Plowright: The problem arises in areas which have a lot of wildebeest, primarily East Africa or the Masai lands. These areas have periodically heavy losses to MCF. Sometimes farmers have to withdraw cattle from the best grazing land because of the high risk of MCF to their cattle. The disease risk simply puts another nail in the coffin of the wildebeest in areas where fencing is present. Wildebeest are a dominant part of the ecology: the migration of the wildebeest in the Serengeti Plains is one of the great zoological sights of the world. It may be difficult to reconcile the interest of these 2 species. Because the white-tailed species of wildebeest are on the endangered list, the loss of wildebeest would be on the world's wildlife scene a disaster of the first order.

Dr. Crandell: What could be done to stimulate interest in MCF outside of the present group?

Dr. Castro: Dr. Crandell, in answer to your question, the laboratories in the U.S. involved with research on MCF have been rather limited. We have tight restrictions in working with wildebeest-associated MCF, and no restriction on sheep-associated MCF.

Dr. Heuschele: In the U.S., the U.S.D.A. regulates and restricts imported ruminants; U.S. born ruminants can move anywhere with impunity. We can sell them to cattle ranchers.

Dr. Plowright: Your evidence in the U.S. is that the normal method of transmission in wildebeest is **vertical.**

Dr. Heuschele: There is also **lateral** transmission from baby to baby wildebeest leading to 100% infection by 5 to 6 months of life. But, what about tolerance in sero-negative babies?

Dr. Plowright: There is no evidence of tolerance in wild populations of wildebeest.

Dr. Heuschele: In order to work with the WC-II strain of MCF, the USDA requires our laboratory to meet requirements of a P3 isolation facility and we had to get a permit to acquire it. We also have isolated other MCF-associated herpesviruses from other species of 3 subfamilies of Bovidae.

Dr. Plowright: The hartebeest virus cannot be transmitted to cattle. However, the hartebeest virus of Reid and Rowe, by cross-neutralization is little removed from the wildebeest virus. Thus, are these the same herpesviruses or different? How are regulations to be drawn up in terms of control of transmission of MCF? One cannot ignore the wildebeest or the wildebeest calving season. In parts of Africa, you have wildebeest and sheep-derived MCF and the conditions under which the MCF occurs are still unknown. You can control the movement of exotics better than potentially infected sheep.

Dr. Heuschele: Both domestic and exotic sheep and goats have also been found to be sero-postive for MCF. We have isolated a virus from a clinically ill Siberian ibex which is a caprine. The ibex had a virus neutralization titer to MCF (Alcelaphine) of 1:8. This virus is a herpesvirus but is not completely characterized at this time. Once we have culled our sero-positive wildebeest, what about sero-positive exotic Mouflon sheep or Cretan goats, which show a high prevalence of antibody to MCF. Species in the family Hippotraginae (oryx), also have high antibody prevalence to MCF.

Dr. Crandell: What I was addressing is: what information has been given to the importers and game ranch owners?

Dr. Heuschele: I've sent letters to exotic game breeders and numerous cattle breeder's organizations to present seminars on MCF.

Dr. Castro: Little has been done to disseminate information on MCF to these groups.

Dr. Plowright: It seems that the problem is the sheep-associated disease because you have a lot of cattle in the U.S., whereas in the United Kingdom and New Zealand it is excessive losses in red deer ranches to MCF. These deer are more susceptible to the sheep-associated form of MCF based on extensive published evidence. The sheep-associated form of MCF is widely disseminated in sheep populations especially in countries where there is restriction of importation of exotic species.

In England, farming of red deer as meat is being encouraged and they have had outbreaks of MCF in some of their experimental herds. Reid and colleagues transmitted the disease into rabbits. They were able to cultivate T-lymphocytes of rabbit origin which produced MCF in rabbits so they called these T-cells NK or natural killer cells. But, these workers were unable to identify a virus so they postulated that the viral genome was in a reduced form but capable of producing disease with no serological conversions. They have also isolated NK cells from cattle afflicted with MCF. Furthermore, these NK cells can be propagated serially on feeder layers and are capable of producing MCF in rabbits and deer. However, rabbit NK cells will not produce disease in cattle. Based on immune-precipitation, these workers believe that some of the proteins of sheep-associated virus are identical to those of wildebeest (*alcelaphine*) virus. The first evidence for a herpesvirus in sheep-associated MCF came from Rossiter's work on sheep sera from Australia in 1969-1970. He found low levels of virus neutralizing activity in sera from sheep associated with outbreaks of MCF in cattle. Thus, Reid and coworkers, in England, have made the major breakthrough in MCF research in the past decade.

Dr. Burton: The Oklahoma City Zoo had some research goats, 12 of 14 which came up sero-positive for MCF by ELISA, and Dr. Metz had only 1 with a seropositive (1:4) SN titer. The zoo program requires that an animal which goes off grounds has to be tested and put in isolation for 1 month and tested for a month before it can leave.

Therefore, to enhance our testing program for exotics at the zoo, does the ELISA correlate with the SN?

Dr. Castro: It did in our studies at the San Diego Zoo, but not 100%. At OADDL, none of 150 random sera from cattle have tested sero-positive by ELISA for MCF. But we still will have to ascertain that both assays are measuring similar antibodies.

Dr. Heuschele: The indirect fluorescence test for MCF lacks specificity but can be used as an initial screen.

Dr. Plowright: The antigen used in the serological test are important, otherwise you are going to be left with a group-specific test which cannot differentiate between herpesvirus from wildebeest, oryx or sheep (if one exists in sheep)!

Dr. Worley: Unless one can engineer a specific viral polypeptide or glycoprotein to coat the ELISA plate to distinguish antibodies to sheep-associated or wildebeest-associated disease, one cannot ever distinguish between the two forms. I think cross reactions between viral epitopes will exist thus making this a difficult task. However, if you had monoclonal antibodies against certain viral epitopes, you could pull out specific viral epitopes by using anti-idiotype monoclonal antibodies. These viral antigen should be specific.

Dr. Heuschele: By SN, I'm not calling titers of 1:2 significant. On titers by SN of 1:4 or greater we had a correlation with the ELISA of 93%. The alcelaphine species in the family Bovidae had the greatest prevalence of antibodies to MCF. In the caprine, we found a 31% prevalence but low SN titer, which is my justification for saying that the caprine MCF virus is a herpesvirus.

Dr. Castro: Our binding data in the ELISA suggests that all viral isolates of MCF are not antigenically the same so Dr. Worley's remarks on differences in epitopes and Dr. Plowright's comments on variations of MCF viruses might be well taken!

Dr. Heuschele: What about the nomenclature of viral isolates of MCF? Ludwig names these viruses by family origin, Roizman by subfamily.

Dr. Plowright: I was the first to suggest to the ICTV committee to name these herpesviruses by subgroups to give some impression as to what are the major hosts or maintenance hosts. I think precise indication of maintenance hosts are the simian herpes-viruses which use the subfamily names. If you are logical, you would adopt the system where the subfamily of the major natural hosts is the name (adjective) by which you name the virus.

Dr. Castro: Dr. Evermann of Washington State University in a letter to me points out the problem in nomenclature, and the use of either Roizman's or Ludwig's classification. (The conferees voted unanimously to use the subfamily name *alcelaphine* herpesvirus in all their publications.)

Dr. Plowright: Eventually the ICTV will decide what classification should be used.

Dr. Castro: Dr. Evermann raised another point regarding the etiology of sheep-associated MCF. He has advocated for the presence of 2 viruses in this disease, one border disease of sheep or BVD virus, and a bovine herpesvirus type IV. Dr. Storz of Louisiana State University feels that in MCF we are dealing with only a herpesvirus.

Dr. Heuschele: Antibodies against alcelaphine herpesvirus-1 do not neutralize virus in the *Movar* or type IV bovid group such as DN599.

Dr. Plowright: I think we are dealing with a group of closely related herpesviruses with MCF which will require specific names in the end.

Dr. Metz: I have found a one way cross with sera to DN599 and MCF antigens; however, no cross reactions by an indirect immunofluorescence test (IFAT) occurred between IBR, PRV and BHMV antisera and the WC-II and Indian gaur isolates of MCF at a 1:20 dilution of serum.

Dr. Whitenack: When in pregnancy does the wildebeest fetus get infected?

Dr. Plowright: It's only a proportion of fetuses, I've isolated virus from fetal spleen and also neonates which became viremic a few days after birth. Therefore, we say those neonates viremic within the first week of life are almost certainly congenitally infected. There is no evidence of neonatal disease or immune tolerance to viral antigens in neonatal wildebeest because all adults develop active antibodies which persist for life.

Dr. Castro: I'd like to start this afternoon with a discussion of the problems of diagnosis of MCF. Three laboratories in the U.S. have been responsible for the collection of most of the serological data: the SDZ (Dr. Heuschele), the National Veterinary Services Laboratory (NVSL), (Dr. Metz), and the OADDL (Dr. Wan). Dr. Wan will first present her data on interferon which may temper our discussion as to the pathogenesis of MCF.

Dr. Wan: Because the virus in these studies was highly cell-associated, we measure the activity of interferon on MCF virus by the production of fluorescent foci units (FFU) in bovine fetal kidney cells. Isolates of MCF virus from an Indian gaur, a Greater kudu and two wildebeest neonates were highly sensitive to two different types of interferons, one from MDBK cells, another from bovine macrophages. The reduction of viral FFU was 50% or greater for the 4 viruses. Also, we found the FFU produced by the WC-II and gaur viruses were reduced by 50% using both human and bovine alpha interferon. By our ELISA for measurement of antibodies to MCF, we found 100% correlation between SN, ELISA and IFAT on sera from 14 wildebeests. In another study at NVSL, cattle with clinical MCF and seropositive by SN for MCF were also seropositive by ELISA; however, there was poor correlation with the IFAT. We also tested serial bleedings from a calf inoculated by Dr. Metz with the WC-II virus. We found by ELISA antibodies to MCF in

the calf using as antigen a nuclear extract of MCF virus. After 12 days, we saw a rising titer in the MCF virus-inoculated calf and we detected an anamnestic response at day 23 after a booster viral dose. The SN data on this calf also correlated with the ELISA. By the ELISA, we also picked up seropositives to MCF in sera from domestic goats. We feel that goats carry a herpesvirus that is similar to the alcelaphine herpesvirus.

Dr. Castro: We also tested cattle sera from outbreaks of MCF in Colorado which were provided by Dr. DeMartini from Colorado State University. We picked up some seroconversion on certain pre and post labeled sera but we do not have the histories on these animals. Dr. Wan has some additional data on sheep inoculated with the alcelaphine herpesvirus type 1 (Greater kudu isolate).

Dr. Wan: After finding goats seropositive to MCF at the SDZ, we obtained 3 Dorset lambs (4 months old) which were seronegative. We inoculated one with the nuclear extract MCF antigen (used to bind to ELISA plates), another with MCF virus infected BFK cells, and one was left as a contact control. Serial 2 day bleedings were tested by ELISA using a threshold of 0.15 absorbence units as a sero-positive reaction. The 2 lambs responded immunologically to the viral inoculum in 6 days. An anamnestic response was seen at 18 days after a booster was given. The sheep receiving infectious virus associated with the cells also responded serologically but the sera produced had lower absorbence by ELISA. The contact lamb remained seronegative.

Dr. Castro: Dr. Metz has questioned whether the greater kudu isolate is similar to the WC-II virus; we don't know. However, we have some preliminary evidence to suggest that there are antigenic differences between viral isolates of MCF. It appears that we are measuring a group specific antigen by the ELISA but one with specific epitopes of MCF virus.

Dr. Wan: This study also shows that anti-bovine IgG conjugates can be used in ELISA for MCF as well as anti-sheep IgG conjugates when sera from either exotic, ruminants or sheep are tested.

Dr. Metz. As an investigator in a diagnostic laboratory, my research was to develop new techniques to diagnose MCF. We did indirect fluorescence using the Indian gaur virus of MCF which we adapted to Vero-MARU (Green monkey kidney) cells. I then received the WC-II virus and grew it cell-free in bovine turbinate cells. I started doing SN and have tested over 1000 sera, mostly cattle. In the IFAT, bovine sera give a nuclear fluorescence but reference MCF serum produces a nuclear and cytoplasmic fluorescence. I think it may be a non-specific herpesvirus cross.

Dr. Plowright: I think you have to take into account age and histories; better a smaller number of sera from a defined group.

Dr. Metz: What I hope to do is not establish prevalence of MCF in cattle but to use the data for comparative purposes on 3 serological assays for MCF.

Dr. Plowright: I did conclude once that cattle with MCF which did not die in the clinical course of the disease developed neutralizing antibody late in the disease. However, later we learned that they certainly developed IFA antibody early on in the course of viral infection.

Dr. Castro: One of the monumental problems in diagnostics is to fit data from animals submitted from practitioners or clients to the laboratory. To obtain actual data, we need to inoculate experimental animals and identify viral-caused lesions and measure serologic responses, but the infancy of research on MCF in the U.S. only began in 1981.

Dr. Heuschele: In 1979, that was the first date for the isolation of MCF virus in the U.S.

Dr. Castro: Dr. Osario formerly of Iowa State University has already shown by restriction endonuclease cleavage the WC-II virus is different by several DNA fragments from the gaur isolate of MCF.

Dr. Hutt-Fletcher: You may pick up differences in restriction enzyme patterns but it may not be significant in terms of calling it a different virus.

Dr. Plowright: I agree.

Dr. Castro: Therefore, to acquire the prevalence data Dr. Plowright has suggested, what do we demand of these serologic assays for MCF?

Dr. Plowright: There are few animals which survive MCF by the alcelaphine herpesviruses. Rossiter has failed to find evidence of a subclinical infection by the alcelaphine herpesviruses. We have never come across cattle which are absolutely refractory to infection amongst the many hundred; it could easily amount to 1000 experimental cattle.

Dr. Heuschele: Is there a geographical incidence of European sheep-associated MCF?

Dr. Plowright: It is easily recognized when there is a close admixture of sheep and cattle. So it has been known in Germany and Switzerland in the winter where cattle were housed in the same stalls with sheep. Since housing conditions for animals have improved, the Swiss and Bavarians now see nothing of MCF. In the United Kingdom, the disease occurs in the Northwest in Scotland where sheep and cattle contacts are most likely to occur, particularly at the time of lambing. In Australia, a sheep flock which initially transmitted MCF to cattle for the first few years, ceased to transmit disease to cattle for unknown reasons. There must be many infected sheep where no transfer of virus takes place. The factors which determine the transfer of MCF are far more important than arguing about differences in serological terms. There has to be a cell-free virus excretion somewhere to get transmission. That is really the fundamental epidemiological question!

Dr. Heuschele: We must also consider domestic goats as potential carriers based on seropositive SN data.

Dr. Plowright: Agreement is universal, that the majority

of multiple cases of MCF in cattle are attributable to contact with sheep. Close contact with sheep is not apparently essential for transfer of virus so another unidentified means of transfer of MCF may exist. The goat is a possibility!

Dr. Castro: There are 3 serologic tests for detection of antibodies to MCF; the SN, the IFAT and ELISA. I think we all agree that the SN should be the final definitive serologic test.

Dr. Burton: Is the ELISA a feasible alternative to test animals?

Dr. Castro: We must first answer these questions on serologic tests so data can be presented to regulatory agencies to establish regulations for the movement of animals and for disease prevention and control.

Dr. Heuschele: In regards to prevention and control, I felt that any animal that had neutralizing antibody to MCF had to be considered a carrier of the genome in some form in its body, because it's a herpesvirus. However, it is my impression that not all these seropositive animals shed the virus to susceptible species. I believe the regulations should require testing of alcelaphine species since the only outbreaks of MCF which have occurred in susceptible species have been in association with wildebeest, sheep and goats. Thus, these are the species that should be tested, and furthermore allow only breeding of seronegative animals. I would not place MCF seropositive wildebeest on game ranches either. Also, I think a 1:8 SN titer to MCF virus is indicative of a seropositive animal. Anything less is equivocal. If we put 100 $TCID_{50}$ of virus in the SN test, and the sera is 1:4 and the ELISA is positive then the animal should be called a seropositive.

Dr. Plowright: I think a 1:4 SN is a good arbitrary level. But, you have to use one strain of virus since SN titers will vary depending on which viral isolate you use.

Dr. Castro: What about cattle-to-cattle transmission?

Dr. Plowright: I should probably think that cattle-to-cattle transmission never occurs from sick to healthy cattle, but one cannot swear to that.

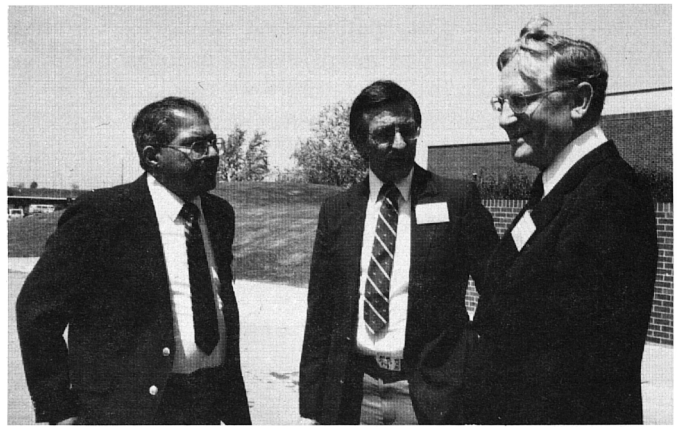
Dr. Castro: We have evidence that MCF did not transfer by contact between MCF-infected deer and a contact deer over a 45 day period.

Dr. Plowright: But, there is no evidence to indicate that these species (cattle and white-tailed deer) shed virus that can infect contacts.

Dr. Heuschele: Harry Anthony of Kansas State University believed he had cattle-to-cattle transfer of MCF but it was never proven.

Castro: At the present time these documented outbreaks of MCF in cattle have not proven cattle-to-cattle transmission.

Dr. Burton: I have to immobilize ruminant animals 3 times for serological testing prior to their leaving the zoo premises. This is excessive for **high risk hoof stock!** Can we bleed them once, screen by ELISA and have seropositives tested further by SN then ship the animal out?



Left to right: Dr. Castro, Dr. Heuschele and Dr. Plowright.

Dr. Heuschele: I'd be willing to say just test alcelaphine and caprine by SN and ELISA.

Dr. Plowright: I think it unfair that we should regulate the movement of species which have never been proven to produce MCF. We know about the wildebeest so you go by the rules according to what you find out. If someone finds transmission from other species like topi, hartebeest, only then should one control the movement of these species.

Dr. Castro: I think the proposed regulation is being interpreted that any animal seropositive for MCF is potentially dangerous.

Dr. Burton: If the 2 forms of MCF exist, does the serology distinguish between both?

Dr. Castro: No!

Now we get into sheep-associated MCF. We have **never** been successful in the isolation of virus from cases of sheep-origin MCF in our domestic cattle.

Dr. Plowright: The indirect serological evidence indicates that there are cross-reactive antibodies to alcelaphine herpesvirus in sheep sera.

Dr. Castro: We can absorb MCF sheep sera with various viral isolates of MCF and see if this approach can identify specific antibodies to MCF virus. I think the consensus at this meeting is that there is a similar virus in sheep and goats which is related in some way; at least some of the epitopes are similar to the alcelaphine herpesvirus of MCF.

Dr. Crandell: Dr. Castro, do you feel uncomfortable about coming up with a standardized SN for the wildebeest agent? It would help zoos and game ranches.

Dr. Castro: The SDZ and NVSL have been gearing their efforts to standardize the SN test for MCF.

Dr. Metz: We've decided the WC-II virus is the best for the SN test because of the variations in other viral isolates from the United States.

Dr. Heuschele: I make a recommendation that we all use

the WC-II virus for testing all alcelaphine (wildebeest) and that we call a 1:4 SN titer positive using 1.5 to 2.5 logs of input MCF virus.

Dr. Plowright: That seems a reasonable figure for virus input.

Dr. Crandell: What about the time interval to read the SN test?

Dr. Metz: I read at 5, 7 and 10 days.

Dr. Plowright: So that is where, at 10 days, you'll be estimating the input of virus?

Dr. Metz: Yes.

Dr. Crandell: What about the type of cells in the assay?

Dr. Metz: We use the Vero-M cells.

Dr. Plowright: You get better figures if you titrate your virus by 1/2 logs to determine endpoints or titers!

Dr. Metz: Yes, and also if you use 8 replicate wells per virus dilution.

Dr. Castro: I think the standardization of the SN test for MCF is the key to numerous laboratories doing the serologic testing once the WC-II virus is released to these diagnostic laboratories. I believe Dr. Heuschele's comments to test *specific* species of animals for MCF is important for our regulatory people. I believe the ELISA developed by Drs. Wan and Metz, independently, will play a vital role in the screening procedure for the alcelaphine species and has the potential to screen other species. Thus, we have to develop better viral preparations for the ELISA plates.

Dr. Crandell: Dr. Castro, if we have dead exotics and domestic ruminants, do we still make our diagnosis by histopathology or should we be doing something else?

Dr. Castro: My belief is that if the laboratory has the facilities, virus isolation should at least be attempted. If you harvest tissue from an animal that dies 24 to 48 hours earlier, your chances for isolation of virus are dramatically diminished. You should have tissues for virus isolation between 4 to 5 hours after death. Don't discourage individual (practitioners) from sending samples to the laboratory. This will be the only way to find out what is going on in the field.

Dr. Crandall: Is sheep-associated MCF a reportable disease?

Dr. Heuschele: Yes, in some states; no in most!

Dr. Castro: We report it to our State Veterinarian.

Dr. Burton: What about recommendations in testing exotic animals at zoos?

Dr. Castro: I recommend you test blood taken at the first immobilization, hold the animal 30 days, and then the animal can be moved and then bled at the receiving point.

Dr. Burton: Or can we get blood on the shipment day?

Dr. Castro: Yes, I would agree to that.

I'd like to thank all the participants and especially Dr. Plowright for his insight and comments this afternoon. I believe we made some inroads in the understanding of the pathogenesis of this disease. I bid you all bon voyage from Oklahoma.