Exotic Diagnostic Procedures

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It has been interesting this morning to listen to references by the previous speakers to the fact that veterinarians sometimes are offensive. I was impressed with one comment that Dr. Hibbs made about the CBC sometimes being an exotic test. In the subject that I have been given this morning the definition of "exotic" really depends on your repertoire of laboratory testing or diagnostic tools that you use in your particular practice. I will discuss two particular areas.

The first will be that of tests which are not commonly used but may be of importance to you as you expand your repertoire of diagnostic tools. The second area will be that of things that are on the horizon that are not yet commercially available or available in most of the diagnostic laboratories, and yet, tests which are under development and may have a significant impact on your diagnostic repertoire.

I am going to ask you to do something that you have not had to do yet this morning and that is just listen. I do not have slides to present in my portion of the presentation this morning so I will ask for your attention so that we can go through some of these things together.

One of the things that has been only referred to this morning but has not been studied in depth has been the use of profiling in making a differential diagnosis. And, as many of you are aware, there are instruments available today which allow one to do a great deal of diagnostic work with a single blood sample. Nutritional diseases and specific diseases of particular organs lend themselves well to diagnosis by using the profiling method. Now, we have conducted an interesting study in profiling that would be of interest to the beef cattle people in that we have been dealing with predictability of weight gains, using the profiling system. And by examining sixteen different parameters of the blood and serum and then following the animals for a feeding period, we have been able to go back and analyze those particular blood profiles and on the basis of that analysis which was done on 423 animals, for example, we were able to predict those that would outgain the others. The difference in the gain in that predictability group was over a quarter of a pound a day. At the current price of cattle, that particular predictability is economic and could be used to help a feedlot operator feed cattle much more efficiently than is presently being done.

I am going to leave profiling and go on to the fact that in diagnostic work the important thing is to know what to ask for and where to take the sample. Most of you have state diagnostic laboratories. Most of them have a list of tests performed. But in some instances, the practitioners are not as familiar with those tests as they could be. And as a result, things that should be available to them are not.

Now, let me talk about three things this morning that are tests which are available at most diagnostic laboratories or at least should be in the near future. Some of the more progressive diagnostic laboratories today are using the electron microscope in a fashion where it has never been used before. Rather than using it for identification of viruses after the viruses have been cultured, they are using the EM to identify morphologic characteristics of virus particles to aid in the diagnosis. Now, these virus particles are observed in tissue, either taken antemortem or in secretions taken antemortem, or in some instances taken from tissue obtained on postmortem examination. But it makes the diagnosis available in a very short period of time. Now, the reason that I say that this is futuristic and important is because there is today a great push to develop anti-viral drugs. There are many of these drugs that are presently under investigation for use in the veterinary field. One of these is a compound called Virasol which is being marketed around the world in countries other than the United States for human use. This compound, which is a nucleoside analog, has great efficacy against animal viruses. In the near future, this compound or similar compounds is going to be available to you to treat some of the viral diseases of animals. When that is the case, then the rapid diagnosis of the particular type of virus so, that the proper anti-viral compound can be selected, is going to be most important to you. So that is something that is now being done and yet the future use of it is going to have even greater importance to you as a veterinarian on the basis of the new therapeutic measures which will be available to you.

The second thing that I would like to bring out is also a diagnostic test which is of great importance to the dairy practitioners. We have lived with bovine lymphosarcoma for a good number of years and we are familiar with the bovine lymphosarcoma virus and yet, the full impact of the disseminatoin of that virus in our dairy herds really has not been realized except by our purebred breeders. I guess it was brought home to us not too long ago at the university where I am located, at Utah State University, where considerable expense had been put into proving a Holstein sire. The production records of his daughters were excellent and the sire was offered to one of the artificial insemination studs and their requirement was that the animal be tested for the presence of

bovine lymphosarcoma virus. Now, the reason for their asking for that test to be done was the fact that foreign export requirements for semen require that all semen must come from animals which have been tested to be bovine lymphosarcoma virus-free. And in this particular instance, the bull was not free. He has no evidence of the clinical disease, but he does have evidence of viral infection. And as a result, that bull is not acceptable by the artificial insemination studs, and hence, although there is no evidence that the virus can be passed in the semen, we do find ourselves in a situation where here is a bull where daughters are having excellent production records, but no one is really interested in further development of the offspring of that particular animal. So I think it is going to behoove the purebred breeders especially to find out what the status of their herd is as pertaining to this virus. The test is available in some of the state diagnostic laboratories. It is available commercially from two private companies that I am aware of and several of the state universities will also do this test. But I think that as a veterinarian you should be aware of the economic importance of this particular diagnostic situation to better counsel your purebred breeders.

The third area that I would like to talk about is also an area of technique and instrumentation which is going to be greater utilized by the veterinary profession and that is the area of high pressure liquid chromatography. In the past, one of the latest disappointments in the diagnostic area has been the availability of techniques whereby we could analyze for drugs and poisonings. As a result, many times the laborious procedure of going through the techniques that were available to us then to arrive at a diagnosis was so great that by the time five or six months had elapsed and the chemists had done all that they needed to do in order to help make the diagnosis, the problem was long gone. The economic disaster had occurred, the animals were disposed of and there was very little that could be done. But with high pressure gas/liquid chromatography, one can extract materials for specific substances and find the nature of the drug, classify it and actually find out the amount of the drug available. Some of those drugs that one can look for with this particular technique are the steroids, the antihistamines, analgesics, anticonvulsants, antidepressants, tranquilizers, aflatoxins, rodenticides and the pesticides. But one of the important things, I think, is the fact that we can use this for antibiotic detection. I do not know how many of you ever worked with detective devices for antibiotics, but many of them were extremely laborious techniques and required that one work with a particular type of organism and it was an assay organism and you had to take the material and compare it with a standard and it was just an extremely laborious situation. With the liquid chromatograph, one in a very short period of time, a matter of hours, can have quantitation and identification of a particular antibiotic. With the pressure of the FDA on the veterinary profession and the agricultural industry to make sure that we stay within the withdrawal times specified for particular antibiotics, this will offer us an opportunity to screen some of these animals to make sure that we are in compliance with the law.

Now I would like to talk very briefly about some futuristic things. This is not science fiction, but it may border on it.

We are in an age where technology is expanding so rapidly that the application of the present technology really has not been worked out, yet there are many people working on it. In a conference that I attended last December in New York City, there were papers presented on a variety of techniques that were still in the experimental stages. One of the techniques that to me was extremely fascinating was the technique of using near infrared reflectants spectroscopy to analyze for nutritive value of feeds. This technique has been perfected for the cereal grain for moisture and protein and also for some other nutritive substances in the grains. The Canadian government is presently using it as a means of quality control and standardization and also a means whereby they grade their wheat and other grains for sale. One paper that was presented by Dr. Schenk from Pennsylvania was on a concept of being able to use different wave frequencies to analyze forages, and thereby ascertain the nutritive value of the forage. As he got into his discussion it seemed very evident that not only could one ascertain nutritive substances within the forages, but that toxic substances might also be able to be detected in this particular instance.

Now, there are some far-reaching things in that concept. We happen to work at a research station where we have a group of plant scientists. These plant scientists are great people. They are very methodical, very meticulous in what they do, and yet it is interesting that all through the southeastern part of the United States and in Texas there have been introductions of new grasses developed by researchers that only after they have been released for use have they found that there are specific problems in certain animals. We feel that with this research machine the potential exists to be able to screen some of these developing species of grasses and forages that are considered for use and screen them for toxic potential, combine those with animal studies and be a lot further ahead in looking at widespread economic disaster from some error presented by a researcher who did not do all the things that were necessary in order to prove the material safe.

The second area that I want to talk to you about is that of enzyme-labeled antibodies. In 1960 and in the 1970's the radioimmuno-assay has provided us with a very extensive, in most instances, and a very good tool to analyze for a variety of different substances. It has had two drawbacks. Number one, the nature of the label itself, which meant that one had to handle it in a very careful manner and that its disposal was done in accordance with the prescribed methods for radiological health. And the second was that it re-

quired expensive equipment, using a gamma or beta counter. With enzyme-labeled antibodies, what one has is a detection situation where antigen antibody complexes can be made by adding the serum to either the antibody that has been previously prepared or the antigen that has been previously prepared, complexing those with a known enzyme and then adding the substrate for that enzyme to the complex and the action of the enzyme on the substrate can then be quantitated through colormetric methods. In doing this, one ends up with a test wherein the enzyme label is stable over an extended period of time, compared to the relatively short life of radioisotopes, and also where the enzymatic activity is detectable with the readily photometric instrumentation, thereby eliminating the need for gamma and beta counters. Thirdly, sensitivity is a function of the label employed in the assay. And fourthly, the need for the separation procedures that we use in radioimmunoassay can be obviated. Now, you might be aware that during the hog cholera outbreak in 1975 ELA was actually used in this particular outbreak and aided tremendously in being able to stop that outbreak because the time for detection was cut from several hours to just less than an hour. In fact, the present techniques will allow the detection to take place in as little time as 30 minutes.

The other thing is that the enzyme-labeled antibody tests lend themselves to automation so that screening can be done on large populations. They presently are using this particular type of technique to test for trichinosis in the hog slaughtering plants in the United States and the procedure has also been developed to be used for brucellosis testing. There is a wide gamut of diseases that could be detected by this particular technique and research is going ahead very rapidly to help develop the techniques necessary to detect those diseases.

Claude Bernard said that the study of things caused must precede the study of the cause of things. In veterinary medicine, you know, we have been in a situation for a good number of years where we have been studying the cause of things. I think with the new technology that is being made available to us now that we are going to be able to detect the cause of things at a much more rapid rate than we have ever been able to do before. I look forward to being a part of that rapidly advancing technology.

Now, I have not taken all of the time this morning for a purpose. I have invited to share the program this morning, Dr. David Hellend. Dr. Hellend is a practitioner from Michigan and Dr. Hellend has been associated with a most unusual toxicosis in Michigan in dairy cattle and has some very interesting information to pass to you. It has been a classical epidemiological and toxicological investigation that has enlightened most in this particular situation and I would like to give Dr. Hellend the floor for 10 minutes.

Conbribution by Dr. Hellend

I should say by way of introduction and perhaps clarification, I reside in Illinois and I was asked to consult in Michigan on the problem of what is known as a PBB problem.

Since 1973 Michigan livestock owners have reported herd disease problems that related to toxic substances in feedstuff. Subsequent investigation by the Michigan Department of Agriculture, Food and Drug Administration and others, revealed the substance to be within feedstuffs distributed through retail outlets of the Michigan Farm Bureau Services, Inc. Contamination of the feedstuffs occurred from chemicals manufactured by Michigan Chemical Corporation, which is now known as Velsicol Corporation, a subsidiary of Northwest Industries. Evidence accepted in court proceedings has established that contamination of agricultural feedstuffs by polybrominated biphenols, hence the terms PBB, trade-named Fire Master, and a number of other chemicals including halligenated naphthalenes, have occurred as early as May 1973 and possibly as early as 1971 when the manufacture of Fire Master began at the St. Louis plant in Michigan by Michigan Chemical Corporation.

Fire Master has been reported to be composed of various isomers, some of which are optical isomers, in which an isomer composition may vary from batch to batch in a commerical compound. Isomers may display unique characteristics when analyzed by gas liquid chromatography and each is likely to have its own particular disease effect.

By way of objective data from the history, in 1971 Michigan Chemical Corporation manufactured magnesium oxide. Those of us involved in the practice of bovine medicine, particularly dairy practice, are familiar with that. The company, Michigan Chemical Corporation, being a major manufacturer of that product in that area began, in addition to that and other compounds, to also produce PBB. Now, PBB is a commercial product. It is used in environments where high temperature and high temperature tolerances would be required–in and around electrical appliances, particularly of commercial nature, such as transformers, electronic devices, photocopying devices and so forth. Of itself, PBB does not have a direct agricultural application. Then in what is now believed was July of 1973 substitution of PBB for MGO occurred at the Battle Creek plant of Michigan Farm Bureau Services, Inc. Then in May of 1974 the feed company, to its retail outlets, to the people who have the contact with the farmers and dairymen, made the announcement. This gap has been widely discussed over cracker barrels in various feed stores. Some people say it is like a gestation, although at seven months it is more like an abortion. In June of 1974 the first 34 herds were quarantined. So this again represents somewhat of a gap, but things are beginning to speed up. In February of 1975, 286 herds with an estimated 37,000 head were reported contaminated, but at levels less than 0.3

parts per million. This figure has significance in that, early on, this was what had been considered as a toxic level. Early on, it was felt the level in an animal, whether milk or meat, that would not be significant. The animals at levels above 0.3 parts per million were considered contaminated. They were so marked and after much legal and environmental hassle, a large area in the federal forest area near Calcaska up near Travor City, about 30 miles east of Travor City, was selected as a burial pit and it was filled and completed in 1976. There were 18,000 cattle, 3,500 swine, 1,200 sheep and a million and a half chickens that were buried in that pit. In addition, large numbers of dairy products and feed were buried in that large pit and filled.

The next date in my historical chronology I have selected is February, 1977. The first trial, Tocoma vs. Michigan Chemical Corporation and Farm Bureau Services, began. The procedures involved, we are all familiar with condemnation procedures and methods for infectious diseases. By and large this was the first significant precedent for condemnation for chemical diseases and, as a result, many of the regulations, procedures, have been made somewhat after the fact. It was the opinion of many and happens to be that of mine that in this instance the court system, our method of jurisprudence, is probably the best way to resolve the matters of damages to farmers. There are compensatory claims, punitive claims, seem to be best worked out through the legal mechanism.

In May of 1976 I was asked by one of the firms of the farmers' attorneys to study the PBB problem in Michigan. I had spent two days visiting farms, talking with local veterinarians, looking over records and laboratory reports. The animals available were from farms exposed to PBB, although there was no way to establish either initiation or termination of exposure. The farm studies would have variations of reportable levels of feedstuff contamination, milk or body fat levels of PBB residues. Since at least 2.5 years had passed since initial exposure, many herds had greatly changed. Some had only a few head left due to quarantine and disposal, such as that area at Calcaska, although there were burials on individual farm sites if governmental approval was gained. Other herds had been decimated by diseases related to PBB. I decided to necropsy two to four head from each farm and selected animals showing a middle point of disease from the survivors and I planned to necropsy between 60 and 100 head.

We chose the following analytical procedures. First we collected samples of tailhead fat and periorbital fat for PBB assay. Now I should digress a minute and say that one of the major problems we have had is that we do not know what we are testing for. The word PBB is code for hexabrominated biphenol. That is only one of the isomers. That is only one of the different numbers of bromine molecules that would be hooked on this biphenol structure. So, even for assaying a component of the commercial product, we are not at all sure that we are dealing with the one that is indeed causing the lesions. Furthermore, it is common knowledge of anyone working with toxicology much at all, that a compound ingested by an animal is most of the time metabolized. The body begins in some way to try to detoxify it. In some instances the secondary or tertiary metabolic products are more toxic than the initiating ingested compound and we simply do not know. So, we were very concerned about this. Being somewhat wary of the laboratories in Michigan, we submitted these fat samples to Warf Institute in Wisconsin. For the comment made about the Ralston 900 Lab, I just got a letter in the mail last Friday that Ralston 900 has bought out Warf Institute. I guess they sent me that because I had been one of their better customers the last year and a half. We did not find any correlation between PBB residue levels in these fat samples and evidence of disease. No correlation. Number two of our analytical procedures, histopath examinations of all major body tissues. Our principal findings were lymphoid hyperplasia and thymic subinvolution. Whole blood was collected for hemograms. Statistically we could not find any abnormalities. Clotted blood for serum was collected for thyroid profiles, no abnormalities noted. Bovine chemistry profile, 12-profile kit, somewhat like Kent was talking about–again no significant abnormalities. Serum protein electrophoresis–we anticipated changes in globulin levels, but the lack of changes suggested that humoral antibody was not involved. The necropsy lesions, while there are piles and reams of data, summarized consisted of predominantly thymic subinvolution and generalized enlargement of lymphatic tissue and the high incidence of acute and chronic infections.

As the number of farms visited grew to 15 and then to 40, my conversations with the dairymen and my observations of their operations revealed, in my opinion, good management practices, good rations and most significantly, no real changes in operation prior to exposure. The only real difference was the history of exposure to PBB.

A summary of herd histories as compiled by me over the last two years would be as follows: Starting in the fall of 1973 and on into the winter of 1973-74 the feed is often impalatable. The cows would throw up their heads. And since most of the feed was fed in the parlor, this would be easy for the farmers to observe. Energy balance problems developed. Cows did not peak in production normally. Cows would go off feed and others would rapidly waste away. In a few weeks' time they would go to skin and bones and they would die in a couple of weeks. Breeding and reproduction problems hit hard. Uterine infections increased and they did not respond to routine treatment. Occasionally, reabsorption at about 40-60 days occurred. Calfhood problems became rampant. Many herds of 80-150 head reported 80-90% calf mortality. Some stillborn, others born weak, dying later after all manner of desperate efforts to save them. By the spring of 1974 the dairymen were alarmed. Most had

been in the business for years and neither they nor their veterinarians had seen anything like it. Laboratory reports from the state usually suggested malnourishment, starvation or emaciation. When cows were put on green pasture in the spring, the farmers were hoping that would change it. Or the young stock being put on pasture. But the problems did not cease. The problems of locomotion became more evident. Ataxia, muscle weakness, joint disorders, listlessness, and lameness, frequently due to elongated hooves prevented usual movement. Both dairymen and attending veterinarians complained of diminished response of therapy. Cuts, bruises, infections lingered in spite of the usual therapy. Abscesses were frequent.

Moving on into the fall of 1974, getting on toward winter, and Michigan has some dandy winters, the problem continued. As the weather got colder the dairymen noticed increased feed consumption, particularly in roughages. No weight gains or increased production accompanied it. This of course lowered feed efficiency. Chronic effects continued and still continue. Since PBB is fat-soluble, I think that is a key thing to understand the effect on dairy cattle. The symptoms seemed to be suddenly aggravated when body fat is mobilized. Just after calving is probably a good example. Cows occasionally go into a rapid wasting-away syndrome. If they do not die in a couple of weeks, they linger on in such a sick state. They hobble around in a weak condition and certainly do not produce milk. I call this a time-bomb situation since cows can be fairly good from the time of their exposure and then abruptly begin to waste away.

Chronic long-term effects are still noticeable in many herds, even where the production is all right. Young stockers become stunted. Teeth show mottling which we interpret is due to irregularities in enamel production. Hooves grow rapidly and abnormally. Alopecia hair loss, especially in the neck, occurs and the skin is thickened at those points. Hair along the withers and along the back is often long, wirey and may have a red tint. Of course, we are dealing with Holsteins almost exclusively. Milk production is erratic. The rolling herd average is lowered. First-calf heifers do not even come into milk frequently and mature cows drop down to between 20-40 lbs. per day, after two or three months in the line.

Analytical data indicate continued exposure. A feed sample collected in August, 1977, contained 1.8 parts per million. Two new contaminated farms have been discovered since October 1 of 1977. This has been as a result of lowering of tolerance levels and a relatively thorough method by the authorities to test all cattle. But worst of all, the owners of contaminated cows have seen enough cows waste away they fear that more time bombs will be walking through their milking parlors some morning.

The descriptions I have made for the last several minutes fit this herd history and to considerable extent fit this individual's history. She had been doing quite well until after she calved, then had less lactation and rapidly began to fall off in production. Now, as I am sure most of the speakers preceding me recognize, you can throw up a picture of a cow and there she is and you cannot say that much about her. You know we have all seen cows that look off and that is about all I can say for this one. But I think, while the previous speakers have talked about specific disease diagnosis and Dr. Van Kampen has talked about some of the larger aspects of diagnosis, we recognize that in these kinds of problems you cannot simply walk up to a cow and say, yes, she has PBB. You need a very exhaustive kind of investigation. The classic kind of problem-solving methods the first speaker was referring to. These things are far more important than walking up to a cow in a free stall and saying she has PBB. About the only things I would say here is she looks like a poor-doing cow and that is about all.

There is another one. This is on the Floyd Jones farm. Again, you are not going to see a lot wrong with her, either, no matter how hard you look. Here is a little fellow. You have seen a lot of these I am sure. If on a farm call you were to see these, yes, he is going to die and he was about to. We finished the process. But this farm had had a very good management history. They had had a very high success rate with their calves. And since PBB exposure they have been losing between 70-90% of their calves no matter what they do. I selected this little guy not only for the pathetic look that he has, but also to point out, as we will see on the next picture, what goes on here.

Now again, you and I have all seen hoof abnormalities from time to time, but in this problem, this is a uniform and highly consistent complaint of these farmers. They will trim them, they will have the vets trim hooves, they will have professional hoof trimmers in, or guys in the neighborhood, and come right back, no effect upon the problem.

Decreased production, high calfhood death rate, the adults wasting away (this is a very mystifying aspect of this disease for me), poor breeding performance, lameness, long hooves, stunting, increased spontaneous disease, poor treatment response, poor hair skin condition, epiphora and mucous rhinitis are common.

We feel that the immune system is related. We do not know how or why, we are certainly hoping that a lot of people can do research on this kind of disease condition. But the immune mechanism seems to be paramountly involved. In the mesenteric lymph nodes we did find increased mass of the entire lymphoid structures and, microscopically, lymphoid hyperplasia was frequent. In the spleen, the Malphigian areas, the white pulp areas are prominent. Again relating to the lymphoid system, on the Peyer's patches there are nodules; not that dramatic but representing hyperplasia. This is a finding we are not at all sure of, but we did see an increased accumulation of mineral substance and macrophages of the lymph nodes. We are calling it hemosiderosis but we are not sure.