Bovine Leukemia Virus Infections—A Continuing Cause for Concern

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It is not the intent of this presentation to provide a lengthy or comprehensive discussion of bovine lymphosarcoma (leukosis) or bovine leukemia virus infections because this was done by Dr. Miller at the 1982 meeting of this organization and published in the April 1983 issue of Bovine Practitioner. An excellent discussion of the clinical aspects of bovine lymphosarcoma, authored by M. Stober, appears in the November 1981 Bovine Practitioner. It is our intent, instead, to discuss those aspects of bovine lymphosarcoma which, in our experience, seems to be a cause of continuing concern and confusion for cattle producers and, sometimes, for veterinarians as well.

At the outset, as a review, the following basic facts regarding bovine leukosis should be recalled: a) Bovine leukemia virus (BLV) is considered to play an eitiologic role in only the adult enzootic form of lymphosarcoma; the calf, thymic and skin forms of lymphosarcoma are not considered to be virus related. b) BLV infections are persistent and, along with the concommitant antibody responses, are maintained for the life of the infected animal. c) There is virtually no free virus present in persistently infected animals, the viral information is present in a subpopulation of the circulating lymphocytes and is expressed in the form of infectious virus when these cells are cultured *in vitro*. d) Because of its cell associated nature, infection is transmitted with some difficulty and usually relies on the movement of infected cells from animal to animal, perhaps most commonly associated with prolonged close contact, biting insects or through the intervention of man (bleeding needles, dehorning, ear tagging, etc.). e) Sensitive and specific serological tests are available to identify infected cattle and have been used successfully in BLV control and eradication programs.

With these basic facts in mind we will now proceed to discuss four major subjects that are repeatedly brought to our attention during conversations with individuals having herd health problems which may, or may not, be related to bovine leukemia virus infections.

Lymphoproliferative disease

It has long been recognized that both persistent lymphocytosis and lymphoid neoplasms are associated with BLV infections. This fact has led to the use of lymphocytotic indexes or "keys" to identify herds infected with BLV. These keys were used with some success in control programs before the more sensitive and specific serological tests were available. It is now generally accepted that approximately 30% of the cattle infected with BLV develop persistent lymphocytosis, that the predisposition to develop persistent lymphocytosis is under genetic control and that persistent lymphocytosis is a benign response to BLV infection and not a pre-malignant phase or early stage of lymphosarcoma. The development of the true neo-plastic or lymphosarcomatous state is a much more rare result of BLV infection. In a study conducted in France the tumor rate among BLV infected cattle was estimated to be 0.31% per year. Some of the animals with tumor (approximately 65%) may have a true leukemia due to the presence of neoplastic cells in the peripheral circulation, a condition quite different from persistent lymphocytosis. Tumor masses in external lymph nodes or in retroorbital tissues are usually readily indentified but if these superficial tissues are not involved the animals may pose difficult diagnostic problems. In these cases a negative serological test for BLV would be helpful in eliminating a possible diagnosis of lymphosarcoma but, because of the prevalence of BLV infections and the low oncogenic potential of the virus, a positive serological test is of only limited value. Apart from the persistent lymphocytosis and lymphosarcomatous conditions described above, there is no evidence that BLV causes other lympholiferative diseases. Thus, in diagnostic situations involving individual animals, or herds, with widespread lymphadenopathy it is, on the basis of present knowledge, prudent to consider etiologies other than BLV infection.

Immunoincompetence or immunosuppression

It is not unusual for us to receive inquiries regarding the potential immunosuppressive effect of BLV. The common situation is that a dairy herd is experiencing a variety of problems including mastitis, metritis, abortion, poor production and a general unthriftiness. In attempts to identify possible causal factors sera are collected and submitted to a diagnostic laboratory for a variety of tests. Almost invariably, as would be expected because of the prevalence of BLV infections, a number of animals are found to be BLV infected and questions concerning the potential immuno-suppressive effects of the virus are raised. It is our opinion that many of these occur because the veterinarian is aware of the profound immuno-suppressive effects of feline leukemia virus infections and suspects that

there may be an analogy in the bovine system. The identification of a retrovirus as a possible etiologic factor in acquired immunodeficiency syndrome (AIDS) in humans may stimulate further speculation regarding the potential immunosuppressive activity of BLV. There is, however, no evidence that BLV is immunosuppressive in cattle. Direct tests of the immune responses of BLV infected and non-infected cattle to viral, bacterial, erythrocyte or toxoid antigens has failed to identify differences between the two groups. Studies of delayed hypersensitivity responses and cellular responses to mitogenic stimulation indicated that the BLV infected cattle reacted as well, or slightly better, than the uninfected control group. In examinations of less specific parameters such as incidence of mastitis, reproductive efficiency or production variables, no significant differences have been found between BLV infected cattle and their uninfected herdmates. It thus seems unlikely, on the basis of current information, that BLV alone plays an important immunosuppressive role in cattle. If BLV plays some type of a cofactor role and in concert with other agents induces immunosuppression, this remains to be described and defined.

Importance of BLV Infections in International Trade

This area seems to be the single most important factor in stimulating interest in BLV infections among U.S. cattle producers. Because many areas of Europe are free of BLV infection, import regulations forbidding the movement of BLV infected animals into European Economic Community nations have been promulgated. Similar regulations have been adopted by a large number of other countries, often regardless of the absence or presence of BLV infection among their indigenous cattle population. Furthermore, because of the problems associated with the seroconversion of exposed cattle during transit or shortly after arrival at destination, there have been moves to require that animals not only be seronegative but that they originate from seronegative herds as well. This often places the U.S. producer in the unfortunate position of either having to sacrifice a significant number of valuable seropositive animals or abandon a segment of the potential market for his breeding stock. To date, a satisfactory solution to this problem, has not been achieved. Regulations regarding the importation of semen from infected bulls are similar but could be more amenable to solution because there is little evidence that normal semen, which is not heavily contaminated with blood cells, is likely to cause BLV infections. The situation regarding the importation of embryos is somewhat similar to semem. If embryos are collected from BLV infected donors it is very likely that the blood present in the flush fluids will contain infected lymphocytes but there is no evidence to indicate that the embryos themselves are BLV infected. Therefore with both semen and embryos it may be possible to institute testing programs that will satisfy both the exporting and importing interests without exposing BLV negative populations to inappropriate risks of infection. The situation regarding the movement of cattle will undoubtedly require additional efforts and perhaps some compromise by both sides if significant obstacles to international trade are to be avoided.

Potential Human Health Hazards Associated with BLV

The repeated expressions of concern regarding potential human health hazards associated with exposure to BLV or BLV infected animals indicates that this is another topic of continuing interest to cattle producers. This interest is perhaps stimulated by results of some (but certainly not all) epidemiological studies which report a higher prevalence of leukemia, or other cancers, among farmers than in the general population. BLV has not, however, ever been isolated from human sources and serological surveys of cancer patients, farmers, veterinarians, laboratory workers and similar "high risk" populations have failed to identify BLV-specific antibodies in a single individual. The subject may receive additional attention because comparisons of BLV and the recently identified human T-cell leukemia virus at the molecular level indicate that there is some relatedness between these agents. The relationship is sufficiently distant, however, that the viruses do not share antigenic determinants. It is therefore generally accepted that the common molecular sequences are evidence of a common evolutionary origin of the two viruses and not an indication of interspecies transmission. Thus the current status of the situation remains, as before, that there is no direct scientific evidence that BLV can infect humans and thus no significant body of knowledge that would incriminate it as a potential human pathogen.

In summary, with the obvious reservation that new findings may, at any time, alter our concepts regarding BLV infections, the current status of our knowledge leads to the conclusion that, except for the frank neoplastic disease and the restrictions on international trade, BLV does not play an important role in cattle disease and has no recognized zoonotic potential.

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Questions & Answers:

Question: What do you think of the complement fixation test?

Answer: It's the most worthless one we have ever invented. It has a lot of false positives and a lot of false negatives. There are state labs that run it, here in Iowa at the University they run it, and there is a private laboratory called Allied Laboratories, in Ames, that runs it. That particular laboratory runs all tests for paratuberculosis, whether it is culturing, or gel difusion, or Elisa.

Question: How do you prevent it?

Answer: What I recommend, at least for dairymen, is to take the calves away from the cows immediately at birth. If possible, give them pasteurized colostrum, and put them on milk replacer. Completely separate from where adult animals have been, or where there has been any manure. Some people are dealing with it by using hutches, and disinfecting the hutches between calves, and putting fresh, uncontaminated soil where the hutches are going to be, between every calf. One large dairy that we worked with followed that program with the exception that they did give one day of colostrum. I followed them for five years after they started that program, fecal cultures every six months, and examining the intestinal tissues at slaughter, and I never found another infected animal that was raised that way. In the Wisconsin program, which most of the states are adopting, they use that same recommendation along with vaccination. The vaccination is basically a way to get their arm behind their backs so that they have to go on to the better husbandry program. Well, there is no particular safe period. You can infect an adult animal. But what we have recommended in the past is to keep them separate until they go into the milking line. The susceptibility is greatest at birth. If you put just one or two bugs in there they seem to find their way into the tissue. They migrate right into the intestinal epithelial cell, mostly down near the ileocecal value, and within an hour you can find them within the mesenteric nodes. So it is very rapid when they're young. It takes an awful lot more to infect a six-month-old animal. Even adult animals have been infected, but I've never heard of any of them becoming clinically ill, if that was their first exposure.

Question: How do you disinfect?

Answer: The only thing more effective than the phenol is 70% alcohol. That's pretty expensive. We use the amphyl, which is Ortho phenylphenol, and a very heavy detergent concentration. I can't tell you the answer to that but usually within 2 5 months, if they have gotten a heavy exposure, they will show a delayed hypersensitivity. That may go away, either because they're getting over it, or because they are going to die from it. And it usually does go away eventually. The ELISA test becomes positive in 4 or 5 months, again depending upon exposure level. I don't think there is a time when you can say they could not have picked it up and be disseminating it. They probably would never get sick if you exposed them at that age.

Question: Could they become shedders?

Answer: Yes, they could have been. We've infected 6-month-old animals and they do become shedders. One of the biggest contamination problems is mold. And the farmers I have dealt with get the notion that if the mold is mixed in evenly so you can't see it it is no problem. Those mold spores come right on through in the manure and there is no effective way to get rid of it. The only thing you can do is tell the farmer to quit feeding moldy feed for a week before he takes the samples. And that is what we have told them in the past. I tell them to begin with not to do it and we get moldy samples; we'll repeat those one time. And if we get them again, he is off the program because he doesn't have to feed moldy feed. That is the only major contamination program we've had. Has that been yours too? (Yes) Okay.

Question: How do you diagnose it?

Answer: If you've got an animal that's clinically ill, the quickest, and not very sensitive method, but the quickest thing you can do is a gel diffusion test. Kits can be purchased or you can send them into the lab for gel diffusion. In this private laboratory that I was telling you about, they charge $2 a specimen for gel diffusion. The ELISA, if you want to find out what the herd situation is and you want to find out how many infected animals there are, you can have the ELISA test done, and I believe that is $3 a sample, $3 or $3.50, something like that. For the farmer's benefit, frequently, even though it is more expensive for the test, the fecal culture is the best because that will tell him which animals are actually shedding, and he can get those off to market. It can also tell him how heavily they are shedding. If he's got a high number and can't afford to get rid of all the shedders, he can get rid of the worst ones. I worked with one farmer not too far from Ames who, the first time we cultured his herd, a third of them were shedding. So we indicated to him how heavily they were shedding. He started by immediately getting rid of the heaviest shedders and then working his way through. By the time we cultured the next time he had got rid of almost all of the shedders he had at that time and there were only a few additional ones

that had started shedding by the second test.

Question: In a herd, two years and up, that were all negative, what would you tell the owner? Where would he proceed from there?

Answer: Well, I'd keep an eye on it, at least with an ELISA test. In fact, I worked with one herd in that situation too, and they had purchased that animal about 6 months before it broke. And it had exposed other animals in the herd. So I recommended ELISA test done on his herd and try to disinfect where that animal had been, clean it up. And getting across what disinfection means is really difficult. Again, another farmer who happened to be a beef man, right on the edge of Ames, had a friend bring two cows over to his place to keep them there while he was being evaluated for DHIA. One of them broke while it was there. He had had it confined in a small metal building while it was there, and I went out and looked at it and told him he'd better clean it up. Well, after that he had put another calf in there and it had some kind of viral disease, I don't know what. But it broke with clinical Johne's disease at 8 months of age. And so he was scared. I told him to get it cleaned up and not put any animals in it. The next time I went out he had cleaned it up. He had taken his front loader out there and scraped it out and that was cleaning it up. I tried to explain to him that if he didn't feel free to eat his eggs off it it wasn't cleaned up. The next time I went out he did have it cleaned up. He really had it polished. And I told him still don't put any animals in it because he didn't need the space that bad anyway. These are hardy little bugs. They'll live for years in the environment. So unless you clean them up and get them out of there and disinfect the buildings, anything that the cow or calf can eat off of or chew on, you aren't going to get rid of it. Goats are particularly bad because of that, because they never quit chewing on everything in sight. So even an adult goat will be going out there and infecting himself off the side of a wall.

Question: What role do wild ruminants play in the transmission of this disease on dairy farms?

Answer: It depends on the level of wild ruminants in the area. In the Northeast where the deer are particularly heavy I am sure that they do play a part because I know from experience that they graze on the same land as the cattle. Here in Iowa I don't think there's very much of that, except on the river valleys. Out in the Rockies the big horn sheep, and so forth, have it. But there does not seem to be too many cattle in with those sheep out there, and goats. But where they are together, they can certainly cross transmit. There was a report last year from Connecticut where it was originally picked up in the deer. Then they chased down where those deer had been mingling with the cattle and found that that herd was infected. Now what infected what I don't know! But the herd and the dairy were both infected.

Question: Dealing with international movement of cattle, when a country is requesting a Johnin test, what degree of confidence can be have in the Johnin test?

Answer: None. It gives some false positives because of other infections. Cattle seem to have a fairly heavy load of *Microbacterium phlei* and other microbacteria that don't cause any disease. But they cause the animal to become hypersensitive. You can deal with that in a serologic test, but we can't deal with that in a Johnin test. On the other hand they can become energic so that no matter how heavily infected they are the animal is negative. That is only a little better than a CF test which is useless.

Question: What do you recommend with the fecal material from a contaminated pen where an animal has been?

Answer: Putting it on row crops, if possible. But not on pastures. We did have one situation in Maine where a company that makes antibodies had a large goat herd and they had been distributing the manure to local gardeners for use in their gardens, and the people found out that they had Johne's in the herd, and they got a city ordinance against them distributing it anywhere. So they called me wanting to know what to do about it. They had been storing it in concrete bunkers while they tried to figure out what to do. The first thing I had them do was check the temperature, and down in those bunkers there was enough heating going on that it was killing the organisms. And they did culture from down in there and were negative. The surface was still infected, but down in where it was hot, it killed them.

Question: It's pretty expensive to send samples in to culture. Would it be possible to culture in our own practices?

Answer: Sure. You can buy the media ready made, but if you're going to hire somebody to do the work I think it's about as simple to send the samples in. I don't know where you're from but this private lab in Ames is $7 a sample when it is a whole herd sample. I believe the University is $10 or something like that. It varies all over the place. Wisconsin was charging $4 or $4.50, something like that.

Question: I'm from Northeast Iowa. We've sent several samples down to you. We've got a few herds now that you are working with. But I just wondered what was the cheapest.

Answer: I've been ordered to stop that. We are competing with private business. We have to stop it.

Question: Is the disease infectious enough that farms downstream should be concerned?

Answer: I would think there would be a small concern, but the dilution factor there would probably take care of it. The main thing is where the calf is nursing from a cow that has layed down in it. That is the biggest source of infection. The next biggest is when they defecate into the water supply. But, again we had one beef owner who had an infected herd and the people downstream from him were concerned. They had not had any problem but they were afraid that they would have. I could not guarantee that their calves might not pick it up, because it is obviously going to move downstream and it is not going to die.

Question: What's the relationship between Johne's and mastitis?

Answer: Animals that have subclinical infections have reduced immune response in the mammary gland. This has not been published but it will be shortly. But the lymphoblastogenic response of the cells in the mammary gland is drastically reduced and within the same herd, the reason given for culling animals that we check at slaughter was five times higher in those that did not appear clinically ill. There was 5 times more mastitis than in those that were not infected.

Question: In a herd where you've identified a few clinical animals, talking about large herds of about 500 cows, how much risk is there of spreading the disease by rectal palpation?

Answer: I don't think there's much chance of that. It's a possibility but the adult animal is so much more resistant and wouldn't carry that many and that is not the preferred site of entry. They enter primarily at the terminal end of the ileum. That is the main place of entry. I put massive numbers into the uteruses of cattle at the time of insemination and up to one week at necropsy I could always find the organisms. At two weeks I found it in 2 out of 3. Three weeks, 1 out of 3. And beyond that, I could not recover the organisms at any time. It did not prevent implantation of the ova. The uterus is not a place where you can infect them. Guinea pigs we can

readily infect in the uterus, and it goes all over the body in them, but we could not recover it from any part of the body in the uterus. And I have not tried to infect by running it up the rectum. But I don't think that is a major problem.

Question: If you had a calf that was infected at birth, how big a threat would it be to other calves, say if it was put in a pen with them about 3-4 months of age.

Answer: Infected at birth? (Yes) It's not going to be shedding at that stage, if that's what you're asking. They don't usually start shedding before they are a year old. We've had some shedders 6-8 months old, but usually year olds are the first ones you find.

Question: In some areas of the country manure is harvested from flush systems and fed back to cattle. Can we assume then that we are feeding the organism if we have it in it?

Answer: Yes, sir. It lasts a long time in any system that we have tried it.