Bovine Leukosis and A.I.

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Many cattlemen have seen a cow or two affected with the disease leukosis or lymphosarcoma. Sometimes, enlarged lymph nodes may be seen externally, especially in the flank or in the front of the shoulders. Tumors develop within the body. Body condition deteriorates toward a fatal termination.

USDA's records show that for many years only about 1 in 8,000 carcasses (less than 5,000 carcasses in 1977) were condemned because of lymphosarcoma in USDA inspected slaughter plants. Consequently, in the recent past, little attention has been given to bovine leukosis in the U.S.A. In Europe, however, governmental programs in some countries have been underway for many years with intent to eliminate this disease. Using a "Key" to interpret numbers of lymphocytes in the blood, entire herds of cattle were slaughtered when some individuals were found to have elevated numbers of lymphocytes.

About ten years ago, as research workers began uncovering a new series of facts, veterinarians involved in AI in the U.S.A. began to feel concern relative to leukosis in cattle. Some of the pieces of the leukosis jigsaw puzzle were disturbing:

1. There were a few happenings on farms and other places where people were in contact with cattle or products of cattle that raised questions as to whether there might possibly be more than a circum stantial relationship between lymphosarcoma in cattle and leukemia in humans.
2. It was appearing that the infectious, virous agents associated with leukosis in poultry and mice were transmitted "vertically", that is, from generation to generation within the sperm and/or egg cells.
3. A new virus was being associated with bovine lymphosarcoma.
4. Surprisingly, when new and experimental immunological tests for the new virus being associated with lymphosarcoma were applied to samples of blood serum collected in the north central states from dairy herds,—some herds known to have at some time included cattle affected with lymphosarcoma, and some herds in which lymphosarcoma had never been observed—, a rather high frequency of positive, "healthy" cattle were disclosed in both kinds of herds.

During 1977, 1978, and 1979, several very important research papers have been published that alleviate the obvious concerns over and provide substantial answers to, the obvious questions that derive from the preceding statements.

No Evidence of Human Health Risk

In 1978, in "Bovine Leukosis: Investigation of Risk for Man", Olson and Driscoll[1] have thoroughly reviewed both field and research reports relative to a possible link between bovine leukosis and human leukemia.

For example, from Michigan, it was noted that there was no evidence found of leukemia-lymphosarcoma in 4,108 person years at risk on 98 farms with bovine leukosis, while on 212 control farms there were 7,968 person years at risk with 1 human case of leukemia-lymphosarcoma. From Denmark, it was found 14 years subsequent to contact, that the prevalence of human leukemia in 1,523 persons, who had been in contact with cattle exhibiting high prevalence of bovine leukosis, was no higher than in other parts of Denmark. From Sweden, it was noted that in an area of high incidence of bovine leukosis there was no statistical relationship between human leukemia and bovine leukosis. People potentially exposed to bovine leukosis in their daily occupations (leukosis researchers, veterinarians, meat inspectors) have been consistently negative to laboratory tests for the virus associated with bovine leukosis.

Destruction of the virus in milk by routine pasteurization has been demonstrated.

Olson and Driscoll conclude by saying: "In summary, it appears that human beings potentially exposed to BLV (bovine leukosis virus) do not develop antibodies to the virus that are detectable by current methods. All statistical studies indicate that there is no relation between bovine leukosis and human leukemia. Although a few cursory reports associate the two, the more extensive and detailed studies provide strong evidence indicating that BLV is not oncogenic for man."

Transmission and AI

In 1978, in: "Influence of Sire on BLV Infection in Progeny", Baumgartner, Crowley, Entine, Olson, Hugoson, Hansen, and Dreher[2] provided data concerning bulls used in AI that were determined to be leukosis infected by either immunological or tissue culture tests in the laboratory or through actual presence of lymphosarcoma:

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a. In Wisconsin, they studied progeny of five dairy bulls actively used in AI that were immunologically positive for BLV, as well as, BLV positive on culture of their lymphocytes, and progeny of six dairy bulls sparingly used in AI that were immunologically positive.

Concomitantly, progeny were studied of 22 control bulls negative to immunological tests for BLV.

Progeny of both BLV positive and BLV negative bulls were one year old or older when tested.

The progeny of the eleven infected bulls were located in 33 herds. Of these herds, 24 had been established with one management for an average of 25 years. Lymphosarcoma had occurred in four of the herds.

The progeny of the eleven negative control bulls were located in the same herds as the progeny of the BLV positive bulls. These progeny had neither dam-daughter nor dam-sire relationship to BLV positive sires. They served as both controls and indicators of presence of BLV in the herds.

Of 576 cows for which data on the sire were available, the infection rate among progeny of positive sires was 15.3% and for the progeny of negative sires the rate was 18.9%. These and other data are not consistent with transmission through male sperm cells. There was no statistical difference in number of positive progeny among five of the BLV infected sires.

b. In Wisconsin, 18 young males sired by four bulls immunologically positive for BLV, were tested for BLV when between 5-21 months of age; 12 were again tested 12 months later. All BLV tests were negative.

The 18 dams of these young bulls were tested for BLV. Two were found BLV positive.

c. On 50 farms in Sweden, 81 daughters of 2 bulls that had been found lymphosarcomatous were studied. Ninety-one herdmate controls having no relationship to these progeny or to their sires were selected on the same farms matching ages and breeds.

Three daughters of one lymphosarcomatous bull were BLV positive and no daughters of the other lymphosarcomatous bull were positive. Nine of the matched controls were positive.

In summary, these researchers state: "Male germ cells appeared to have no influence in causing BLV infection in progeny in the three studies. Each of the three studies provided contributing evidence," and "Three serological studies indicated no transmission of BLV infection to progeny sired by artifical insemination from bulls infected with BLV."

In addition, in 1979 in: "Infectivity Tests of Secretions and Excretions from Cattle Infected with Bovine Leukemia Virus", Miller and Van Der Maaten[3] reported inoculating entire semen ejaculates from each of 8 bulls with persistent BLV infections into 8 sheep with replicate injections of semen from 4 of these bulls made after a five month interval. None of the sheep developed antibodies.

Nevertheless, in 1977 in: "Susceptibility of Cattle to Bovine Leukemia Virus Infection by Various Routes of Infection", Miller and Van Der Maaten demonstrated BLV infection in four of six mature cows following instillation of infected leukocytes (white blood cells) into their reproductive tracts. The material inoculated consisted of 1-2 ml quantities of leukocytes concentrated from 40 ml of whole blood from an infected donor cow placed into the cervical canal of each test cow. The dose of lymphocytes placed in their cervices in these experiments was approximately 640 to 6400 times greater than equal parts of whole blood and semen processed for AI.

Competent processors of frozen semen know that presence of blood or pus in semen is cause for discard.

How Bovine Leukosis Transmission Does Occur

Early workers believed that bovine leukosis spreads principally, but not exclusively, "horizontally", that is, postnatally by contact with infected animals. In 1978, in: "Natural Mode of Transmission of the Bovine Leukemia Virus: Role of Blood-sucking Insects", Nielson, Piper, and Ferrer[4] reported that in their experiments, non-infected cattle became infected during summer months while maintained in contact with infected cattle. Non-infected cattle did not become infected during winter months. Engorged horseflies that had recently fed on a BLV infected cow were found to contain in their midguts millions of BLV infected lymphocytes. It is well known that many varieties of blood-sucking insects are capable of transmitting many different diseases. However, the transmission of BLV infection by insects has not yet been confirmed experimentally.

In 1977, in: "Susceptibility of Cattle to Bovine Leukemia Virus Infection by Various Routes of Exposure", Van Der Maaten and Miller[5] reported transmitting BLV experimentally when as few as 2,500 washed lymphocytes, (the number present in only .0005 ml of bovine blood) were injected into the skin.

Present thinking seems to agree that horizontal transmission by blood transfer into the skin is a probable route by which susceptible cattle become infected. Blood sucking insects may be transmitters of this disease. Blood contaminated instruments, inoculation of blood for premunition, or therapeutic transfusion of blood, can be means of transmission.

Diagnosis of Bovine Leukosis

In those rare instances when infection with the virus of bovine leukosis progresses to formation of tumors, called lymphosarcoma, diagnosis is made upon clinical examination or necropsy and is confirmed by microscopic examination of a piece of the tumor.

Hematological Keys

Until a few years ago, the usual approach to diagnosis of leukosis was counting lymphocytes in the circulating blood and, then, interpreting this number through the use of a

"key" which allowed for normal, age-related variations. In Denmark, employing the "Bendixen Key" on a herd basis, entire herds considered leukosis-infected were slaughtered, consistent with government sponsored eradication programs. Some other European countries implemented programs similar in principle.

Regrettably, the "Bendixen Key" method, which can have value when judiciously applied on a herd basis, came into too wide use—or misuse— for diagnosis of leukosis in individual cattle, frequently as an import requirement of governments.

Today, the limitations of hematological keys when applied to individual cattle are very clear. Ferrer *et al*[1] point out that since . . ."60% or more of adult cattle actively infected with BLV do not have persistent lymphocytosis, peripheral blood counts (hematological keys) are not suitable for the diagnosis of BLV infection." Olson *et al*[7] reported that Bendixen Key interpretation of total lymphocyte counts were evenly divided between positive, suspect, and normal in 64 serologically reacting cattle, in which virus presence was demonstrated, all of which were located in multiple case herds. In one group of about 65 mature dairy bulls used for AI, 12 were serologically positive at a time when only one of the 12 bulls was interpreted by Bendixen Key as positive. This exceptional bull subsequently became Bendixen Key negative.[8]

In light of the present evidence regarding validity of "key" interpretations for diagnosis of bovine leukosis in individual cattle, it appears reasonable to expect that future official demands for conducting or submitting of results of such "key" tests for individual cattle should cease. Not only do "key" interpretations produce a majority of false negatives, but, likewise, some false positives are obtained because lymphocytes may increase for non-leukosis retalted reasons.

Immunological and Other Methods—

Several immunological test mechanisms have been developed which involve recognition of antibodies in blood serum produced specifically in response to infection with BLV. Immunofluorescent antibody, agar-gel immunodiffusion, radioimmunoassay, complement fixation, and virus-neutralization antibody tests have been employed.

The virus of bovine leukosis can be demonstrated by culture of lymphocytes and examination with an electron microscope or by a radial gel test and, also by production of syncytia in a culture of certain foreign cells.

These advanced test techniques are providing accurate evidences of BLV presence in cattle. Further improvement of and applications for these procedures are developing rapidly.

Bovine Leukosis and Lymphosarcoma

Lymphosarcoma, which is the progression of leukosis virus infection to tumor formation, should be considered as the visible tip of the leukosis iceberg. It is now apparent that only a very small percent of cattle carrying the leukosis virus ever develop lymphosarcoma. Typically, BLV infected cattle appear and remain healthy.

In 1978 in: "Comparison of Production Variables of Bovine Leukemia Virus Antibody-Negative and Antibody-Positive Cows in Two California Dairy Herds", Langston et al[9] studied two herds of dairy cattle of 100 and 367 head, in which 70% and 60.2%, respectively, of the cows were antibody positive for BLV. In summary they stated: "With regard to variables of age, milk production, and reproductive efficiency, BLV-infected cows had no greater mean age, no lower milk production, and no lesser reproductive efficiency on the average than did non-infected cows."

Only a very small percent of BLV-carrier cattle ever develop disease. Heredity, stress, and age may be among the presently unknown factors determining if and/or when lymphosarcoma develops. Clinical lymphosarcoma nearly always progresses to termination.

How Common Is Bovine Leukosis?

In a survey of 7,000 cattle conducted by University of Wisconsin researchers in the north central states, reactors to immunological tests were found in 2/3rds of 100 herds of 4,400 dairy cattle and 1/7th of 50 herds of 2,800 beef cattle. In another survey the same workers, testing about a thousand cows in 11 selected herds, found 222 reactors. Five herds with no cases of lymphosarcoma for 13 to 33 years had 2 to 16% reactors while 6 herds with 24 cases of lymphosarcoma in the preceding 7 years had 24 to 42% reactors.[10]

Other researchers have reported 0 to 86% of 651 cattle to be serologically positive in twelve dairy herds in Pennsylvania and New Jersey and 0 to 90% of a sample of 20 cattle to be serologically positive in each of fifteen beef herds in Mississippi and Texas.[11]

A serological survey (non-random) involving 1,295 cattle in 74 herds located in Georgia, Pennsylvania, New York, Texas, and Wisconsin revealed 28.2% positive reactions among the dairy cattle and 2.6% positive reactions among the beef cattle. There were 52.0% positive reactions in herds with a history of bovine leukosis as compared with 13.7% positive reactions in those herds with no history of leukosis.[12]

In one herd of two dairy herds studied in California 70% of 100 cattle were serologically positive to immunological tests for leukosis is surprisingly high. For the beef cattle population, the percent positive may be lower; but, the incidence of positives in specific beef herds may be very high.

What Is Being Done/Can Be Done About Bovine Leukosis

In Europe, a high level of official concern continues regarding bovine leukosis. Their attitudes derive from their clinical experiences with lymphosarcoma, and originated prior to knowledge that lymphosarcoma is just the tip of the leukosis iceberg. Programs to control and eradicate bovine leukosis are of long standing, are committed to continue, and, possibly intensify through utilization of the latest

diagnostic techniques.

In the U.S.A., there are no official regulatory policies or programs in respect to bovine leukosis. Today, only within a small community of veterinary scientists, is bovine leukosis virus infection recognized as being characterized by wide prevalence, high incidence, and very low mortality. It may be said to be a "disease" that, preponderantly, isn't a "disease problem".

To the present, most herd managers are ignoring bovine leukosis virus infection. Nationally, as well as in most leukosis-infected herds, potential economic loss from lymphosarcoma is small and may not justify major effort or expenditure at either governmental or private levels.

In the U.S.A., in the present and near future, bovine leukosis will probably be of most importance in herds that sell cattle to countries requiring evidence of leukosis-free status for the herds of origin and for the individual cattle submitted for exportation/importation.

Summary and Conclusions

1. **There is substantial present evidence that there is no public health risk from bovine leukosis. There is no present evidence that there is public health risk from bovine leukosis.**
2. There is substantial present evidence that bovine leukosis is not transmitted by AI. There is no present evidence that bovine leukosis is transmitted by AI.
3. Present evidence suggests that:
   Transmission within herds is principally by close contact. Blood sucking insects, and blood contaminated intruments that transfer bovine leukosis virus in blood may be responsible.
   Transmission between herds is by introducing the virus of bovine leukosis within new, healthy-appearing cattle that harbor the virus.
4. **"Key" methods of diagnosis of leukosis** for individual cattle by interpretation of lymphocyte counts are unreliable and may be used fraudulently. Normal lymphocyte counts are found in the majority of BLV infected cattle.
   Immunological methods of diagnosis of leukosis using blood serum are reliable; but, there can be a few false negatives and false positives—depending upon method used and stage of infection.
5. Bovine leukosis virus infection that does not progress to lymphosarcoma seems to have no measurable effects upon either milk production or reproduction. In relatively few instances of leukosis virus infection does lymphosarcoma ever develop. Lymphosarcoma is malignant.
6. Surveys indicate that the virus of bovine leukosis is probably present in more than half the dairy herds of the U.S.A. and in more than 10% of the beef herds. The number of infected cattle in infected herds may range from a few to many. Generally, incidence of infection increases with age.
7. **For control of bovine leukosis within herds** emphasis should be placed upon (a) avoiding close contact between leukosis infected and non-infected cattle (b) Reducing possibilities for blood transfer by controlling blood sucking insects and by avoiding blood contaminated instruments, (c) feeding calves colostrum and/or milk from leukosis negative cows, (d) raising calves and replacement heifers separate from infected cows.
   For control of bovine leukosis between herds emphasis should be placed upon avoiding introduction of new cattle which originate from leukosis infected herds. Individual new cattle must be demonstrated repeatedly to be free of the virus of bovine leukosis.
   Presently, as well as in the past, many leukosis positive bulls are/have been in regluar AI service with no evidence of semen-borne transmission of leukosis.
   For control of bovine leukosis domestically and in export/import. In light of the high incidence of the virus of bovine leukosis in the cattle population of the U.S.A., and in consideration of the fact that the best present evidence indicates that bovine leukosis is not transmitted by AI, it follows that semen can be received into herds with much greater security than can live animals.

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