Bovine Leukosis — Its Importance to the Dairy Industry in the United States

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ABSTRACT

Bovine leukosis describes lymphatic cancers of cattle. The most common form of this disease occurs in adult animals and is caused by bovine leukemia virus. Infection is widespread in the United States, especially in dairy cattle, but the virus produces tumors in only a small percentage of infected animals. Nevertheless, bovine leukemia virus has been receiving attention from the dairy industry because of its importance in health certification of cattle or semen intended for export. Another source of concern is whether bovine leukemia virus poses any risk to human health. These problems are discussed in the light of recent technological advances in tumor virus research and specifically regarding our current understanding of the biology of bovine leukemia virus.

INTRODUCTION

In the last few years a disease known as bovine leukosis has received attention in the United States. Our intent in this presentation is to define and describe the disease, summarize research findings that have led to our current understanding of its etiology and pathogenesis, and assess its current and potential importance for the dairy industry.

DISCUSSION

Definition and Description of the Disease

Leukosis is defined broadly as a proliferation of body tissues that produce white blood cells or leukocytes. When we speak of leukosis in cattle, however, we actually are referring to only one type of leukocyte, the lymphocyte. Because the basic pathology of bovine leukosis is a cancer of lymphocyte-forming tissue, other terms that frequently are used synonymously are lymphosarcoma and malignant lymphoma. The disease also has been called bovine leukemia, but this term is not pathologically correct for the disease as it occurs in cattle. Leukemias are cancers that primarily affect the leukocytes in blood, and although some leukotic cattle do have an abnormally high number of lymphocytes in their blood, it is only a secondary effect that occurs subsequent to development of solid tumors in one or more organs of the body.

According to a historical review by Bendixen (7), bovine leukosis was described first in Germany in 1878. By the early 1900's several reports had been published that indicated in some herds the disease was enzootic; i.e., it was constantly present but occurred in only a small number of animals. Later, in the 1930's, statistics obtained from meat inspection reports provided evidence that the disease was especially prevalent in several districts of northern Germany. Meat inspection records also showed that leukosis was in the United States at that time, but there was little information available from other countries to indicate the distribution of the disease.

The early clinical investigations of leukosis in Germany that resulted in recognition of the disease as a distinct pathologic entity also led to a belief that animals with subclinical disease could be detected by blood examination. This idea was based on observations that in herds where leukosis was enzootic, many of the healthy cattle had higher numbers of lymphocytes in their blood than did cattle from unaffected herds. German scientists developed numerical hematologic standards by which such cattle could be classified as “leukotic”. Although these “leukosis keys” were a cumbersome and crude tool for research purposes, investigators used them to provide useful basic knowledge of the disease. Results from numerous epi-
demographic and experimental studies convinced researchers that there were several clinical forms of leukosis in cattle but that only one of them was transmissible. The contagious form was designated enzootic bovine leukosis, and other types of lymphatic cancer were grouped together under the general name of sporadic bovine leukosis.

The three types of lymphatic cancer included in the sporadic category are known as calf, thymic, and skin leukosis. In the calf form, which usually occurs in animals less than 6 mo old, tumors are in virtually all lymph nodes and in many other tissues as well. Thymic leukemia is also a disease of young cattle, but it occurs in a slightly older age group, 6 to 18 mo. In this form, the tumor is not always limited to the thymus, but thymus is the tissue most severely affected. Both calf and thymic forms of leukosis are fatal. Skin leukemia, however, is considered a benign disease. The tumors, which are only in the skin, disappear after a few weeks, and the animal then remains clinically normal. Cattle with skin leukemia are usually between 1.5 and 3 yr of age.

All of the sporadic forms of bovine leukemia are relatively rare, and there is no experimental or epidemiologic evidence that suggests they are contagious. Therefore, we will not discuss them further, and the remainder of this paper will consider only the enzootic or adult form of bovine leukemia.

Enzootic bovine leukosis (EBL) is a disease of mature cattle, the peak incidence of tumor development in animals between 4 and 8 yr of age. A variety of clinical symptoms may be seen in affected animals, depending on which body organs have been infiltrated by tumor cells. A clinical diagnosis is usually not difficult if external lymph nodes are involved or if tumors can be detected by rectal palpation; however, an accurate diagnosis may be extremely difficult if the tumor is restricted to internal lymph nodes or body organs. Besides lymph nodes, the tissues most frequently affected are heart, abomasum, uterus, kidney, spinal canal, and eye. Tumors in these tissues usually are not detected unless by necropsy. The disease is routinely fatal, and if an affected animal is sent to slaughter, the carcass will be condemned.

Etiology of Enzootic Bovine Leukosis

The knowledge about EBL available when Bendixen reviewed the subject in 1965 was accumulated through leukosis keys. The hematologic test was not specific or sensitive enough to allow many firm conclusions, but transmission experiments suggested that a virus might be the etiologic agent of this type of leukosis. Although electron microscopic and virologic studies were encouraging, no convincing evidence of an oncogenic virus had been reported. Then in 1969, Miller et al. found that lymphocytes from many cattle with either tumorous leukosis or lymphocytosis produced virus-like particles if they were cultured in vitro for a few days (42). This finding was confirmed by Dutta et al. (18), Kawakami et al. (30), and Schmidt et al. (62). Although the particles appeared to have a structure similar to certain leukemogenic viruses of other animal species, there was no proof that they represented an infectious agent or that they were involved in pathogenesis of bovine leukosis. In 1972, Miller et al. showed that if calves were inoculated with lymphocyte cultures that contained the virus-like particles, within a few months their lymphocytes developed the ability to produce the same kind of particles (48). Furthermore, several of the calves also developed lymphocytosis. A similar finding subsequently was reported by Schmidt et al. (61). These observations strongly suggested that the virus-like particles seen by electron microscopy in short-term lymphocyte cultures were actually an infectious virus and that they were capable of inducing a clinical syndrome that many people considered to be a subclinical phase of leukosis. In addition, Olson et al. presented evidence that the virus was oncogenic by the experimental induction of tumors in sheep (52). The choice of sheep as an appropriate experimental animal in which to test for oncogenicity of the virus was based on earlier experiments described by Mammerickx (41) and by Wittmann and Urbanek (74). They reported that blood from cattle with lymphocytosis produced lymphoid tumors in sheep within 2 yr after inoculation. This incubation period was much shorter than in similar cattle experiments, and rate of tumor development was higher.

In the same year that the particles from lymphocyte cultures were reported to be infectious and oncogenic, the first studies on viral antigenicity were published. Miller and Olson (43) reported that antibodies to the virus
could be detected by immunodiffusion, and Ferrer et al. (21) also described a serologic test that was based on immunofluorescence. Both techniques demonstrated that sera from many cattle with clinical leukemia contained virus specific antibodies. Subsequent studies, in which these and other methods were used, demonstrated that the bovine virus was antigenically distinct from the tumor viruses of other animals (19, 58, 27, 40). Olson et al. found a higher prevalence of antibody to the bovine virus in cattle herds which had a history of leukosis than in herds which had not experienced tumor (51). This observation subsequently was confirmed by Ferrer et al. with the immunofluorescence test (20), and based on the accumulated evidence for an etiological role of the virus in EBL, they designated the agent as bovine leukemia virus, or BLV.

Characterization of Bovine Leukemia Virus

In the first few years after BLV was recognized, progress in understanding the molecular biology of the virus was hampered by the lack of a suitable tissue culture system for its propagation. Then in 1974 Van Der Maaten et al. reported that BLV could be cultivated in cultures of fetal sheep spleen (71), and Ressang et al. described the establishment of BLV-producing cell lines by infection of fetal calf lung cells (58). Subsequently, Graves and Ferrer described a cell line of infected bat lung cells that also produced large quantities of virus (28). The availability of these culture systems allowed virologists to produce enough material for biochemical and biophysical characterization of BLV.

The work of Dietzschold et al. (14), Gilden et al. (27), Kettmann et al. (33), and Zhdanov et al. (75) showed that BLV contained an enzyme known as reverse transcriptase. This finding was the basis for classifying the virus as a retrovirus, a category that includes almost all the leukemogenic viruses of other species. Reverse transcriptase plays an important role in the molecular biology of retroviruses. After the initial infection of a cell, the viral enzyme catalyzes a cytoplasmic reaction that results in production of DNA molecules that are complementary copies of the infectious viral RNA. The DNA copies then are integrated into chromosomal DNA of the cell's nucleus. Once integration occurs, viral information can be retained indefinitely through replication of cellular DNA during normal mitotic division. The viral genome can continue to code for production of viral proteins, but there is no requirement for the virus replicative process to be completed.

Experiments published by Kettmann et al. (34) and Callahan et al. (11) demonstrated BLV genome in DNA from tumor cells of cattle with EBL. Kettmann et al. later reported that tumor cells from sporadic forms of bovine leukemia do not carry BLV sequences, thus confirming that the virus is causally related only to the enzootic disease (31).

Biochemical analysis of BLV showed it is composed of several proteins that can be identified by molecular weight determinations in polyacrylamide gel electrophoresis. The major internal, or core, protein was found by Gilden et al. (27) and others (13, 39) to have a molecular weight of about 24,000. It now is referred to commonly as the p24 protein. A second important protein, which occurs in knobs located on the surface of virus particles (73), has a carbohydrate moiety and is classified as a glycoprotein. Onuma et al. recognized that this surface glycoprotein was an important antigen of BLV (53), and we then used it to develop an agar gel immunodiffusion test for detection of BLV-infected cattle (44, 45). Many other serologic assays are now available, but the gel diffusion test is the most widely used because of its practicality, low cost, and specificity. The glycoprotein antigen also has been used to prepare an experimental inactivated vaccine for protection of cattle against BLV infection (46). Recently Portetelle et al. (57) and Schmerr et al. (60) described a unique biologic characteristic of this antigen. Unlike most glycoproteins, in which antigenicity is a function of the peptide moiety, the immune response to BLV infection appears to be directed against the carbohydrate component of the molecule. It is possible that this finding may lead to a new approach in our search for practical and effective methods to prevent BLV infection in cattle.

Most of the in vitro studies of BLV have utilized a tissue culture technique that was described by Ferrer and Diglio (22). This test, called the syncytium induction assay, is
based on the ability of infectious BLV to induce fusion of cells in an appropriate culture system. In addition to its usefulness for basic virologic investigations, the assay can be used to detect virus in the blood lymphocytes of infected cattle. It is especially useful for identification of infected calves, because serologic tests cannot be used on animals with antibody obtained from colostrum.

Epidemiology of Enzootic Bovine Leukosis

Prior to isolation of BLV, there was some knowledge about the epidemiology of EBL; however, because the lymphocytosis test was a poor indicator of infection, it did not provide enough information to allow an understanding of many important factors. When the serologic tests for BLV became available, studies could be more exacting. One important question to be resolved was if the primary mode of virus transmission was vertical, i.e., infection of a fetus from its dam or sire, or horizontal, the result of infections passed between animals postnatally. Ferrer et al. used serology to study the epidemiology of BLV in a high-incidence leukosis herd (26). They found that virtually all adult cows in the herd were infected, and, therefore, almost all of the young calves had BLV antibodies from colostrum. After 6 mo of age, as colostral antibody disappeared, most calves became serologically negative. Although a few animals became positive in the next few months, the majority of cattle did not show evidence of infection until they were almost 2 yr old. Ferrer et al. concluded that the management practice of segregating heifers from adult cows probably accounted for the low infection rate in young animals. In another study, Piper et al. described an experiment in which calves from herds with a low incidence of leukemia were foster nursed on cows from the high-incidence herd and then reared in contact with the offspring of those cows (55). The foster nursed calves showed no significant incidence of BLV infection until they were more than 18 mo old, again demonstrating the important role of horizontal transmission in BLV epidemiology.

Although serologic studies show that most BLV infections occur postnatally as a result of contact, the mechanism of virus transmission is not understood so easily. The experiments of Stock and Ferrer (64), Driscoll et al. (16), and Baliga and Ferrer (2) showed that neither virus particles nor viral antigens are produced from blood lymphocytes until after several hours of in vitro cultivation. These findings suggest that transmission between cattle does not occur by way of cell-free virus, and several animal experiments also support this viewpoint. In a study on the pathogenesis of BLV, we were unable to find evidence that a significant amount of cell-free virus is in blood at any stage of infection (69). In other experiments we also failed to find any infectious virus in saliva, urine, or nasal secretions (47). The lack of evidence for cell-free virus in infected cattle is in marked contrast to the reported demonstrations of virus production by blood lymphocytes. From these observations the most logical conclusion is that initiation of a new infection usually occurs after a blood transmission between animals. We have examined some of the most likely routes through which infectious lymphocytes might be introduced into a susceptible animal and have concluded that although infection can occur after exposure of mucous membranes, the skin is a more efficient route of entry (67). If we assume that transmission of blood from an infected animal to the skin of a susceptible animal represents the most significant mechanism of infection, it is not difficult to propose several possible means for this to occur. Bech-Nielsen et al observed a higher incidence of BLV infection in summer months than in the winter, and they speculated that the variability was from greater insect activity during warmer weather (6). They also reported the recovery of BLV from tabanid flies that had fed on an infected cow, but actual transmission of BLV by flies was not demonstrated. Certainly traumatic lacerations and mechanical transfers by needles or surgical instruments also could be suspected.

In addition to contact transmission, it also is recognized that in utero infection of fetuses can occur. Piper et al. reported that 18% of calves from BLV-infected cows already were infected at birth (56). However, these data were obtained in a study of one herd with a high incidence of leukemia. Based on recent evidence that susceptibility to BLV infection is influenced by host genetic factors (9), we suspect that the rate of congenital infection might vary from herd to herd. Ferrer et al. reported a 3% rate of
prenatal infection in calves obtained from infected dams in several farm herds (26), and we have observed an identical rate in calves from experimentally infected cows (70). Some of the cows in our study were inoculated before they were bred whereas others were inoculated at various stages of pregnancy. The infection of a fetus in utero appears to be an entirely random event, because cows that produce a congenitally infected calf subsequently can give birth to uninfected calves (56).

In addition to being the source of a transplacental infection, an infected cow might transmit BLV to her calf through colostrum or milk. Both of these materials contain virus (47, 23), and newborn calves are susceptible to infection by the oral route (67). Because calves of BLV-positive dams also receive virus-specific antibodies in colostrum, we have examined the effect of passive immunity on virus transmission. Our experiments show that colostral antibody is protective against oral exposure to BLV-infected lymphocytes (69). We have concluded that infection through colostrum or milk can occur, but it probably does not happen often because the colostral antibody concentration level is usually high enough to prevent infection by the oral route. This conclusion is supported by serologic surveys that show a low prevalence of BLV infection in cattle under 2 yr of age (51, 26), by the foster nursing experiment of Piper et al. (55), and by the low rate of infection in calves fed colostrum from positive dams and then reared in isolation (25).

In some of the early epidemiologic investigations of EBL, evidence was presented suggesting bulls were capable of spreading the disease by vertical transmission to their offspring. With use of the more precise BLV serologic and virologic techniques, little evidence has been found to support this proposal. We were unable to detect infectivity in semen from positive bulls (47), and Baumgartener et al. (3) could not find any difference in BLV prevalence between offspring of infected and noninfected bulls. Recently, however, a report was published by Lucas et al. describing BLV in semen from an infected bull (37). This work suggests that even though infection through semen probably is not a major factor in the epidemiology of BLV, it cannot be assumed that transmission by this route never occurs. Perhaps of more importance, however, is the potential impact of a bull's hereditary influence on his offspring with respect to their susceptibility to horizontally transmitted infection. In an epidemiologic study of a highly infected dairy herd, Burridge et al. estimated heritability at .48 (9). They interpreted this finding as suggestive evidence for a considerable genetic influence on susceptibility to BLV.

Pathogenesis of Bovine Leukemia Virus Infection

Even during the years when lymphocytosis was the only method for diagnosing EBL, it was recognized that not all infected cattle developed tumors. It was not until serologic tests for BLV were perfected, however, that we realized how infrequently virus infection actually results in clinical disease. Except for the development of antibody, most infected cattle do not show any detectable clinical response.

The results of experimental inoculations and observations in naturally infected herds indicate that about one-third of the cattle with BLV infection develop lymphocytosis (48, 61, 24). Abt et al. demonstrated that this hematologic response was genetically controlled (1). They found that familial aggregations are characteristic not only of lymphocytosis but also of tumorous leukosis. The two clinical phenomena aggregate independently, however, and Abt et al. concluded that lymphocytosis should not be considered a premalignant phase of leukosis. Recently Kettmann et al. published restriction enzyme analysis studies that also support this conclusion (32). They found that in lymphocytosis the BLV-infected lymphocytes are polyclonal with respect to integration sites of the viral genome whereas in leukosis the tumor cells are monoclonal.

Although the biologic nature of lymphocytosis is still not clear, one possibility is that it represents a type of immune response. Muscoplat et al. (50) and Weiland and Straub (72) found that lymphocytosis is the result of an increased number of B lymphocytes, which are immunoglobulin-producing cells. Paul et al. (54) and Kenyon and Piper (35) showed that B lymphocytes are the carrier cells for BLV, but the latter investigators also reported that the expanded cell population in lymphocytosis is composed of uninfected B cells. Because the functional status of the uninfected subpopulation of B lymphocytes has not been investigated,
we cannot draw any conclusion regarding the possibility that they represent a specific type of immune response to BLV infection.

As indicated previously, we now know that the number of BLV-infected cattle that will develop tumor is extremely small. Based on studies in a high incidence herd, Ferrer (24) has estimated that the rate of tumor induction by BLV is less than 5%. Crespeau et al. (12) conducted a 3-yr study in a population of French cattle and found that during that time the rate of tumor development among BLV-infected animals was .84%. With the exception of genetic predisposition, factors that may play a significant role in the progression of BLV infection to tumorous leukosis have not been identified. Hormonal, environmental, and immunologic influences are among the considerations most frequently cited. The complexity of a multifactorial disease such as EBL requires extensive and sophisticated analysis of epidemiologic data. At this time no such studies are available, and this should be a fruitful area for further research.

Current and Potential Impact of Bovine Leukemia Virus

To evaluate the importance of BLV to the dairy industry in the United States, we first must consider what is known regarding the prevalence of virus in this country. The first indication that BLV might be a relatively common infection of US dairy cattle was a report by Baumgartener et al. in 1975 (4). In a serologic survey of approximately 7000 cattle from five North Central states, they found animals with BLV antibodies in 66% of the dairy herds. The infection rate among beef herds, however, was only 14%. This selective concentration of infected cattle in dairy breeds also was reported by House et al., who conducted a survey in five states from different parts of the country (29). The greater virus prevalence in dairy versus beef cattle is not surprising, because several years ago Theilen et al. reported that the enzootic form of leukosis occurs more commonly in dairy than in beef breeds (65).

There has not been a statistically-based serologic BLV survey in the United States, so it is not possible to give an accurate estimate of the overall prevalence of infection. Limited surveys have been done in a few states, and infection in dairy cattle ranged from 13 to 48% (59). The US Animal and Plant Health Inspection Service, which is responsible for a significant portion of the BLV testing for export certification, has reported that between 1975 and 1980 the annual rate of positive sera ranged from 13 to 19% (59). Because the bulk of our cattle export market involves dairy animals, we can assume that the figures apply primarily to that segment of the cattle industry. They probably underestimate the actual prevalence of BLV in adult dairy cattle because most exportations involve young heifers, and serologic surveys consistently have indicated that rate of infection increases with age.

As we indicated in discussion of EBL pathogenesis, the transition from asymptomatic BLV infection to clinical tumorous leukosis is a relatively uncommon occurrence. In some herds, perhaps because of genetic or other undescribed but important secondary factors, the expected tumor rate is greatly exceeded, and then the economic losses may be severe. As an example of the total monetary loss that EBL represents in the US, we can cite the estimate of Sorensen and Beal that the cost in 1978 was approximately 7 million dollars (63). Of course, most of this expense is borne by dairymen because of the predominance of EBL among the milking breeds.

Probably more important to the dairy industry than the direct cost of EBL death loss is the impact of BLV in international trade. For the past two decades many European countries have had EBL control programs. In the beginning, regulations were based on the use of leukosis keys to identify infected cattle or infected herds. The hematologic test was also used to determine that cattle were free of EBL before they could be imported to those countries. The lymphocytosis test lacked adequate sensitivity to detect many BLV-infected cattle, so such restrictions did not interfere seriously with sales of US cattle. However, since the serologic tests have been incorporated into import regulations, our relatively high BLV prevalence severely limits the number of eligible cattle. Furthermore, several countries are proposing to allow importations of cattle only if they originate from BLV-free herds, a stipulation that virtually could destroy the export market for US dairy cattle. Such a loss might
not impact heavily on many individual producers, but for the dairy industry as a whole the value of this market is fairly significant. For example, in 1978 a total of 56,156 dairy cattle were exported, and the estimated value of these sales was at least 145 million dollars (49). The federal government is responding to the current situation by attempting to establish a certification protocol that would allow a group of cattle to be designated as BLV-free without consideration of the herd of origin (59). If foreign countries are willing to accept such a proposal, participation of the US dairy industry in international trade may be preserved.

One aspect of BLV that has attracted some interest in the scientific community is the potential hazard that this virus might represent to public health. One of the first observations that raised this possibility was an electron microscopic study in 1959 by Dutcher et al. in which it was reported that milk from cows in a herd with a high incidence of leukosis contained virus-like particles (17). No proof ever was obtained that these particles were BLV. Twenty years later, however, we were able to demonstrate by sheep inoculation that milk contained infectious virus (47). Ferrer et al. recently confirmed our work and also reported that the infectivity was not in the form of cell-free virus but as virus-infected cells, presumably lymphocytes (23). Baumgartener et al. studied the effect of pasteurization on milk to which cell-free BLV had been added and found that infectivity of the virus was destroyed (5). The effect of pasteurization on virus-infected lymphocytes, however, is unknown.

The possibility that human beings might be susceptible to BLV has been examined in several ways. In one study, before the virus was identified, milk from cows in a herd with a high incidence of leukosis was fed to several different species of newborn primates (38). Two young chimpanzees developed a hematologic disorder and pneumonia and died. Retrospective pathologic studies led to diagnosis of erythroleukemia, a different type of blood cancer than is associated with cattle leukosis. No evidence was presented that the chimps were infected with BLV or that the milk they received was responsible for their disease. We subsequently investigated whether chimpanzees were susceptible to BLV by experimental inoculation and found serologic evidence that they became infected (66). We were not able to recover virus from any of them, however, and there was no evidence that the infection produced disease.

Because chimpanzees are a species closely related to man, the evidence that they produce a typical serologic response to BLV suggests that if human infections occur, they might be detected with the serologic tests currently available. All of the results from surveys of human sera have been negative (10). In several studies, the sera examined included samples from people with known or likely exposure to BLV-infected cattle or milk from such animals.

In addition to serologic surveys, several epidemiologic studies also have been conducted to determine whether there is any relationship between leukosis of cattle and cancer in humans. Almost all of these have been negative (8). One exception is a recent report by Donham et al., who examined several characteristics of human leukemia and the bovine population in Iowa (15). They found that there was a positive correlation between acute lymphatic leukemia in male humans and a high cattle density, especially dairy cattle. The same investigators reported that in counties where bovine leukemia had been diagnosed, the incidence of this particular human leukemia was higher than expected. The Iowa studies did not include determinations of BLV prevalence, however, so it cannot be concluded that the relationships had anything to do with the virus. In fact, they may suggest that in the high incidence counties there was a common environmental factor that not only enhanced the tumor-producing capacity of BLV but also acted as a primary carcinogen in humans.

To summarize our view of the significance of BLV to the dairy industry, at present the primary concern is the high prevalence of virus infection in the US and the consequent limitation on cattle exports. The economic loss from clinical leukosis is less important nationally, but in some herds this is a significant problem. An unknown, but potentially important, factor in assessing the impact of BLV is a possible perception by the public that the virus is an undesirable contaminant of milk. Even though current knowledge indicates that BLV does not present a hazard to human health, media
reactions and public opinion cannot be predicted with certainty.

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