BOVINE LEUKEMIA VIRUS: DEVELOPMENT OF CONTROL STRATEGIES

BY

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ABSTRACT

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The development of strategies pertinent to the control of bovine leukemia virus (BLV) was undertaken in 3 different studies. The roles of environmental sanitation and physical separation of BLV positive animals from BLV negative animals were analyzed via a prospective age cohort analysis in study 1. The prevalence rates and incidence rates of the BLV positive animals were reduced significantly ($P \leq 0.05$) during a 3-year period. The effects of environmental sanitation without physical separation were analyzed similarly in study 2. In the absence of physical separation, the incidence rates increased significantly ($P \leq 0.05$) during a 1-year period, but the prevalence rates did not increase significantly. A determination of the duration of BLV colostral antibodies in dairy calves in Michigan was reported in study 3. Using estimated-weighted regression analysis, the duration of colostral antibodies in 27 calves was 70.88 days. The factors affecting the duration of colostral antibodies and the practical applicability of study 3 were discussed.

To

my mother, Alice Ruth Johnson

my father, Marvin Johnson, and

my sisters and brothers, Edgar

Byron, Cheryl Janice, Michael,

Cynthia, and Kenneth Jerome.

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INTRODUCTION

Bovine leukemia virus (BLV) is the etiologic agent of bovine leukosis. Synonyms for bovine leukosis are enzootic bovine leukosis, leukemia, bovine lymphosarcoma, and bovine malignant lymphoma. Bovine leukosis is a neoplastic disease of cattle that was first recognized by veterinarians late in the 19th century. There are 2 forms of bovine leukosis based upon the frequency of occurrence of the disease. Enzootic bovine leukosis, or the adult form, was classified because of its endemic nature in certain herds and geographic regions. This type is restricted to adult cattle approximately 4 years of age and older. The sporadic form of bovine leukosis occurs more infrequently than the enzootic form. There are 3 subclassifications of sporadic bovine leukosis, and these 3 subclasses are restricted to relatively young age groups. The calf type (juvenile type) occurs in calves up to approximately 6 months of age and is characterized by widespread involvement of all lymph tissue. The thymic type (adolescent type) occurs in animals between 6 months and 24 months of age. The thymus gland is the primary tissue initially involved. The entire brisket region may become involved later.

The skin type occurs in young adults and is characterized by subcutaneous neoplastic nodules which may regress temporarily.

Bovine leukemia virus was discovered by Dr. Janice M. Miller in 1969 (Miller et al., 1969). It is a member of the Family Retroviridae (retrobackwards) because it possesses the enzyme reverse transcriptase in its viral genome (Dulbeco and Ginsberg, 1980, Essex et al., 1980). Reverse transcriptase facilitates the synthesis of deoxyribonucleic acid from ribonucleic acid. Infection with BLV is persistent because the viral genome becomes incorporated into the genome of the host's cells, specifically the lymphocytes (Kettman et al., 1976, Callahan et al., 1976, Essex et al., 1980).

The economic impact of bovine leukosis was first recognized in European countries in the 1950's through the 1970's. The disease became so prevalent that BLV eradication programs in the form of test and slaughter were established to reduce the prevalence of leukosis quickly. The primary criterion upon which the decision to slaughter cattle was based on an abnormally elevated lymphocyte count resulting in persistent lymphocytosis (Ferrer, 1980). Unfortunately, it was unknown at that time that many animals infected with BLV may not have a persistent lymphocytosis. The eradication programs met

with limited success only. More recently, test and slaughter programs have been based on serological tests, specifically the agar gel immunodiffusion (AGID) test. Because the AGID test measures an antibody response to BLV, control and eradication programs have become more efficient. The serologic tests results in fewer false negative animals remaining in the herds to infect susceptible animals.

Though the national impact of bovine leukosis in the United States has not been as serious as it was in European countries, serious potential economic losses do exist. The prevalence of antibodies to BLV ranges from 10% to 48% in dairy cattle and from 1% to 7% in beef cattle in the United States (Olsen et al., 1973; Baumgartner et al., 1975; Burridge, 1982b). The estimated loss due to slaughter of cows with BLV-induced lymphoid tumors was 3.4 million dollars in 1978 (Sorensen and Beal, 1979). The estimated loss due to morbidity and mortality also was 3.4 million dollars during the same year. The economic losses are greatest in herds in which enzootic bovine leukosis occurs and in herds in which a significant portion of the income arises from the export of cattle and semen. The reason for losses due to enzootic leukosis are obvious. The cattle and semen export business is endangered because more foreign countries

are restricting their cattle and semen imports to BLV free cattle only. Thus, the need for controlling the spread of and developing an economically sound eradication program for bovine leukosis becomes apparent.

Paramount to the control of any contagious disease is a thorough understanding of the routes of transmission of that disease. Numerous studies (see Part 1) have shown that transmission of BLV occurs primarily horizontally. These studies have shown that any route which is amenable to the transfer of BLV infected lymphocytes is a possible route of transmission of the virus. Any control measures developed for BLV must include measures which prevent the transfer of BLV infected lymphocytes.

If control measures for BLV are to be developed, it is necessary to determine the effectiveness of these control measures. As with other contagious diseases there are multiple routes of transmission of BLV. Obviously all routes of transmission of BLV are not equally important. A determination of the importance of certain routes of transmission, particularly those that are difficult to institute, is also necessary. A study of such a nature could reveal whether it would be worthwhile to institute certain control measures. Since development of a BLV vaccine would be an important part of a BLV eradication program, it becomes essential to determine the

duration of BLV colostral antibodies in calves. The active immune response initiated by a vaccine would be inhibited if the vaccine is administered when colostral antibodies are present.

The objectives of these studies were: 1) to develop a herd-based control strategy for BLV in a herd with a high prevalence of BLV, 2) to determine the importance of the role of physical separation of BLV negative from BLV positive animals in controlling the spread of BLV, and 3) to determine the duration of BLV colostral antibodies in Michigan dairy calves.

PART ONE

BOVINE LEUKEMIA VIRUS: A HERD-BASED CONTROL STRATEGY

ABSTRACT

A study was conducted to develop a herd-based control strategy for bovine leukemia virus (BLV). Michigan State University's closed lactating dairy herd of 114 cows was utilized for the study. Ninety-five percent (95%) of the cows were positive for BLV antibodies, as determined by the agar gel immunodiffusion (AGID) test in November 1979. To develop the control strategy program, the following management practices were instituted: There was a complete physical separation of BLV positive from BLV negative animals for 3 years after which the two groups were physically mixed. Different and sterile supplies and equipment were utilized for any veterinary medical related activity. Personnel working on the farm were constantly made aware of the transmission of the disease and how to minimize it. A constant vector control program and other miscellaneous management practices were implemented. The BLV seronegative animals were examined monthly for BLV antibodies starting at 6-7 months of age. The reactor animals were separated from the seronegative animals when the reactors had 2 consecutively positive AGID tests. A positive BLV serotest was not a criterion for culling. Following a 3-year complete

separation of positive animals from negative animals, the overall point prevalence decreased from 95% to 34%. The results were further categorized according to 4 age groups of 6-15, 16-23, 24-47 and 48 months and older. The percent BLV positive animals decreased from 19 to 17, 58 to 14, 90 to 33, and 100 to 90 percent for the respective age groups. Following a 10-month discontinuation of physical separation of positive animals from negative animals, there was still a decrease in the overall point prevalence rate. The limitations and practical applications of the program are discussed.

INTRODUCTION

Various investigators have reported different reactor rates to bovine leukemia virus. The percent BLV positive cows in some dairy herds have ranged from as low as 2.1% to as high as 85.4% (Baumgartener et al., (1975), House et al., 1975, 1977). The reactor rates in the House et al. studies were similar to a 1979 study of the dairy herd at Michigan State University (MSU) where 95% of 114 lactating cows were seropositive to the glycoprotein antigen.

In herds in which enzootic bovine leukosis (EBL) occurs, economic losses due to death, slaughter, veterinary services and decreased milk production as cited by Sorensen and Beal (1979), may become severe. We encountered similar losses as estimated by the owners of 3 commercial herds and their veterinarians. Several countries have instituted BLV control programs in which BLV positive cows are not allowed entry. Personal communication with farmers owning registered cows indicates that BLV positive tests may be the only limiting factor in the sale of cows and bulls to member countries of the commission of the European communities (CEC), to some South American countries, and to Canadian bulls studs (Ruppanner, personal comm. 1984). For those registered herds in which exportation is of economic importance and for commerical herds in

which clinical lymphosarcoma is a problem, a practical BLV control program would allow a gradual transition to a BLV-negative status and would alleviate these two potentially detrimental problems of commercial dairies and registered herds. Attempts at control and eradication of any infectious agent must consider the method of transmission of that agent. Any method by which BLV infected lymphocytes may be spread is a potential route of transmission of BLV. Natural transmission of BLV primarily occurs postnatally and horizontally (Ferrer, 1979). Piper et al., (1979) showed that cattle raised in contact with BLV seropositive cows converted from 0% to 100% BLV positive, whereas cattle raised in isolation converted from 0% to 18% seropositive. Blood-sucking insects may play a role in BLV transmission (Bech-Nielsen et al., 1978). BLV-infected lymphocytes were recovered from tabanids allowed to feed on a BLV positive cow in that study. Buxton et al., (1982) injected sheep with the mouthparts of mosquitoes allowed to feed on blood from a BLV-infected cow with persistent lymphocytosis. Positive AGID and radioimmunoassay tests were found after 3 months. Oshima et al., (1981) produced results similar to Buxton's in an experiment in which tabanids collected while allowing them to feed on a BLV positive cow were placed on sheared BLV-seronegative sheep which later became seropositive.

Thurmond and Burridge (1982), and Thurmond et al., (1983), however, showed that no seroconversion occurred in the insect-prevalent summer months, but that it was highest in November, indicating that vector transmission may be less significant. Regardless of the potential method of transmission, Straub (1978) and Thurmond et al., (1983) contend that both donor and recipient must be in a shedding and receptive state, respectively, and that close physical contact is necessary for transmission to occur.

Vertical transmission of BLV may occur postnatally through milk, colostrum, and by dam-to-calf contact and prenatally through an utero infection of the fetus by the dam (Kenyon et al., 1982). BLV was found in whole milk and milk cells of naturally infected, BLV positive cows. BLV seronegative sheep became BLV positive after injection of whole milk and milk cells. Miller and Van Der Maaten (1979) tested milk and colostrum for infectivity in sheep. Sheep inoculated with colostrum and milk became BLV seropositive after 2-3 months and remained so for the 6 month duration of the study. The seropositive sheep were positive for BLV by the syncytial infectivity assay. Straub (1982) performed a similar experiment using oral exposure to milk and colostrum, and produced results contrary to the aforementioned results. Colostral antibodies have been shown to inhibit infection of animals consuming BLV

infected milk, and these antibodies have a duration of 3 to 6 months (Piper et al., 1979; Van Der Maaten et al., 1982; Burridge et al., 1982a).

Prenatal transmission of BLV was shown to be 14% to 18% in a herd specifically bred for susceptibility to lymphosarcoma (Ferrer et al., 1976; Piper et al., 1979). Jacobsen et al., (1983) found that only 3.8% of calves born to naturally infected seropositive dams from 2 herds were seropositive at birth. It would be possible to retain large numbers of seronegative calves, even from BLV seropositive dams, in herds seeking reduction of BLV seropositive cows if these low percentages of prenatal infections are typical of most naturally infected herds.

Excretions and secretions other than milk and colostrum have been examined for transmissibility of BLV. Miller and Van Der Maaten (1979) and Straub (1982) were unsuccessful in producing BLV infections in sheep after intraperitoneal injection of semen, urine, saliva, and nasal secretions into BLV seronegative sheep. Likewise, Kaja and Olson (1982) were unsuccessful in producing infections in sheep after injecting semen from 30 BLV seropositive bulls. Viral particles, however, were recently recovered from bovine semen (Lucas et at., 1980). The important benefit of artificial insemination (AI) is to prevent contact transmission of the virus from BLV seropositive sires to

to dams and vice versa, if natural breeding was practices.

OBJECTIVE

Because of the prevalence of antibody to the disease, its economic implications, absence of chemotherapy and absence of a vaccine, it is imperative that other methods of controlling this disease on a herd basis be developed. Because of increased knowledge about BLV transmission, a control program must be designed to exploit our knowledge of transmission of this disease. The objective of this study, therefore, was to develop a practical program for preventing transmission of BLV in a herd with a high prevalence of BLV seropositive animals and to determine the time required to achieve that goal in this herd. In organizing the program, only those control measures which were considered practical, realistic and appealing to livestock producers were considered. The new guidelines did not affect the previous guidelines for culling cows. Alterations in daily routines were made only if there was evidence that the practice could lead to transmission of BLV.

MATERIALS AND METHODS

Herd Background

Animals. The Michigan State University dairy herd was used to conduct the study. The mature herd consisting of 114 registered lactating cows, was screened for antibodies

to bovine leukemia virus in November 1979, and 108 (95.0%) were BLV positive. The herd was open until that time, but it was closed from November 1979 through January 1983 with the exception of the purchase of 2 additional cows. Closing the herd meant that all replacement animals were raised on the farm. The 2 purchased cows were not screened for BLV prior to purchase. They were assumed to be BLV positive, and were placed in the positive barn. The herd was a multi-purpose herd, being used for teaching of dairy husbandry, animal reproduction, nutritional research, and for milk production. The public was allowed free, daily access, and open-house was conducted at least once annually. Calves were being retained only for replacement of culled cows.

Feeding Practices. All lactating cows were fed by hand using portable equipment. Because the non-lactating cows were housed 1.21 km from feed-storage facilities, the total mixed ration was hauled to them daily. Feed equipment was not disinfected between these facilities. The calves were fed whole, non-pasteurized milk from the bulk tank. This milk was from both BLV positive and BLV negative cows.

Insect Control. Insects were controlled using commerical fly repellents as fly bait (indoors) and in backrubbers (outdoors).

Milking/Calving. All cows were milked in a double-eight herringbone parlor. The BLV positive cows were milked last. The BLV negative cows calved in maternity stalls in the negative barn. The BLV positive cows calved in the positive barn.

Management Changes

To implement the control measures, the following management changes were made:

Housing. Strict separation of BLV-seropositive and BLV-seronegative cows was practiced from November 1979 through January 1983. The original 6 BLV negative and 108 BLV positive cows were placed on opposite ends of the same indoor facility. The 2 groups were separated by 2 wooden barriers, each barrier serving as an entry-port to each group of cows. The lactating cattle were confined indoors except during heat checks and exercising. Neonatal calves nursed their respective dams for colostrum regardless of the dam's BLV status. The calves were separated from their dams at approximately 12 hours postpartum and were initially housed in individual hutches 0.09 km from the lactating herd. The hutches were placed 1.5 m apart. Although males and females were initially handled similarly, the males were sold at 4-8 days of age, and only females were retained. As space requirements for BLV negative

cows increased, they were housed according to body weight and/or age. The cows were separated into 3 groups: non-pregnant heifers (0-14 months), pregnant heifers (15-23 months) and lactating cows two years of age and older. The younger lactating cows were separated from the older cows in the early phases of testing because most of the BLV-seronegative lactating cows were in the younger age group. Facilities for these age groups were 1.21 km from the units housing lactating BLV positive and BLV negative cows. Dry cows were housed in dry lots 0.09 km from the lactating herd. Beginning in January 1983, strict separation of positive and negative cows was discontinued.

Injections and obstetrical practices. Different, sterile, disposable needles were used for each cow when injections were performed. A different plastic, disposable obstetrical sleeve was used to palpate each BLV negative cow. All cows were bred by artificial insemination. There were separate heat check pens for BLV positive and BLV negative cows.

Personnel awareness of the project. The personnel informed of these procedures were the herders, student employees, graduate students and other researchers. These people were heavily involved in the daily activities of the dairy and had intimate contact with all the animals.

Miscellaneous management changes. Dehorning was

performed with electric dehorners. There was no disinfection between calves. Calfhood vaccinations for brucellosis were performed around 2 months of age. The tattoo was not disinfected between calves. Ear tags were attached during the first week of life. No castrations were performed as all males were sold between 2 and 8 days of age.

Testing Schedule

The BLV negative herd was tested for antibody to BLV each month. Initial tests were performed at 6-7 months of age when colostral antibody had become minimal. Blood was collected by coccygeal venipuncture and was allowed to clot for 10-16 hours at 19.0°C. The serum was separated by centrifugation at 420 g, and stored at -20°C until serological tests were performed.

Serological Test Used

The agar gel immunodiffusion (AGID) test for the glycoprotein antigen of bovine leukemia virus, as described by Miller and Van Der Maaten (1976), was used to identify BLV positive and BLV negative cows.[a] Results were interpreted in a darkroom with the aid of a portable 25-watt desk lamp. Seroconversion was defined as 2 consecutively positive AGID tests performed at 6 months of age or later and performed at 4-week intervals. Seroconverted cows, if

lactating, were moved into the BLV positive barn. None of the cows were ever culled strictly because of a BLV positive test.

Analysis of Results

The working hypothesis was that there would be a significant difference between decreases in the herd and age specific prevalence rates and incidence rates when AGID positive cows were physically separated from AGID negative cows, and when sanitary measures which prevent the spread of BLV-infected lymphocytes were utilized. Point prevalence rate (PPR) as defined by Schwabe et al. (1977) was used to determine the magnitude of BLV infections in the herd in January of each year. Point prevalence rate was defined as the number of AGID positive animals divided by the number of animals at least 6 months of age that were tested for antibodies. Examination for significant differences between the PPR of October 1979 and each succeeding PPR was performed using the Chi square test of independence at a probability level of $p \leq 0.01$ (Schefler, 1979). The incidence rate (IR) was used to measure the likelihood or risk of an animal's developing detectable antibodies to BLV. The incidence rate was defined as the number of new AGID positive cases divided by the population at risk during a 12-month period. The population at risk for determining IR included all negative animals in an age group at the

beginning of the year, and the new cases were those animals that seroconverted during a specific year. Examination for significant differences between the incidence rates was performed using the Chi square test of independence at a probability of $p \leq .05$ (Schefler, 1979).

RESULTS

Point Prevalence Rate (PPR)

There was an overall annual decrease in the herd point prevalence rate from 95% in November 1979 to 34% in January 1983 in spite of an annual increase in the number of cows tested (Fig. 1.1). There was a significant difference between the PPR of 1979 and each succeeding PPR ($p \leq .01$). There was a small decrease in the PPR measured in October 1983, but there also was a small decrease in the number of animals tested.

Age-Specific Point Prevalence Rate (ASPPR)

Age-specific point prevalence rates were variable (Fig. 1.2, Table 1.1). The most dramatic decrease in ASPPR was seen in the 16-23 month group and the least dramatic decrease was in the 4-year and older age group. The 6-15 month age group was the only one in which there was a decrease in PPR followed by an increase.

Incidence Rate (IR)

The overall IR (Fig. 1.3) increased between 1982 and 1983. However, there was no significant difference between these rates ($p > 0.05$). Incidence rates for each of the 4 age groups were compared in 1982 and 1983 (Fig. 1.4). The IR either remained the same or decreased in 3 or 4 age groups.

DISCUSSION

The marked differences between the PPR in the youngest age group compared to the oldest age group can be explained by the number of positive cows in each group at the start of the study. The oldest cows were part of the milking herd, and 100% of them were BLV positive on their initial test (Fig. 1.2). There were very few BLV negative cows being added to this group during the study, and there was only a small decrease in the PPR. The youngest cows, on the contrary, had little to no contact with BLV positive cows, recieved the benefits of the control program, and all BLV negative calves were added to this group. The decrease in the PPR reflects these occurrences (Fig. 1.2). Though not sigificant, the reason for the increase in the overall IR between 1982 and 1983 is difficult to determine. This increase may be explained by the fact that there was a 4-fold increase in the number of animals tested in the 24-47 month group in 1982 and 4 of the 7 (57%) total

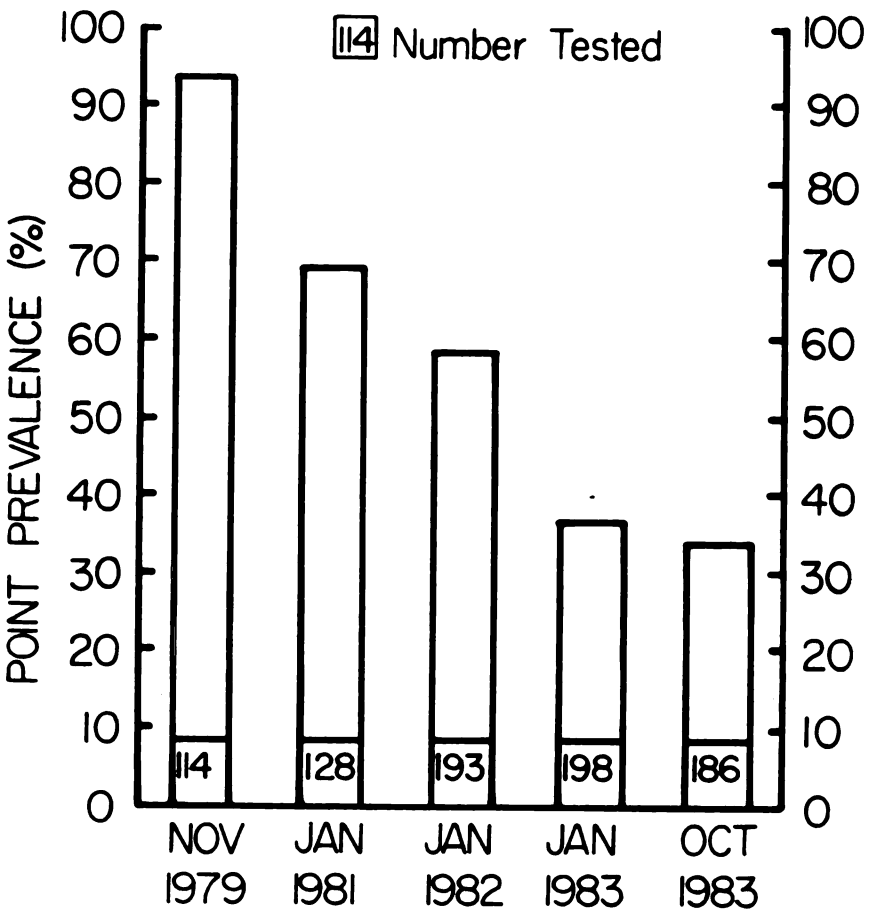

Figure 1.1: Point prevalence of AGID reactors at age > 6 months. One hundred fourteen (114) includes lactating cows only. Increase from 114 to 193 coincides with retention of all seronegative heifers and extends over 2 calving seasons.

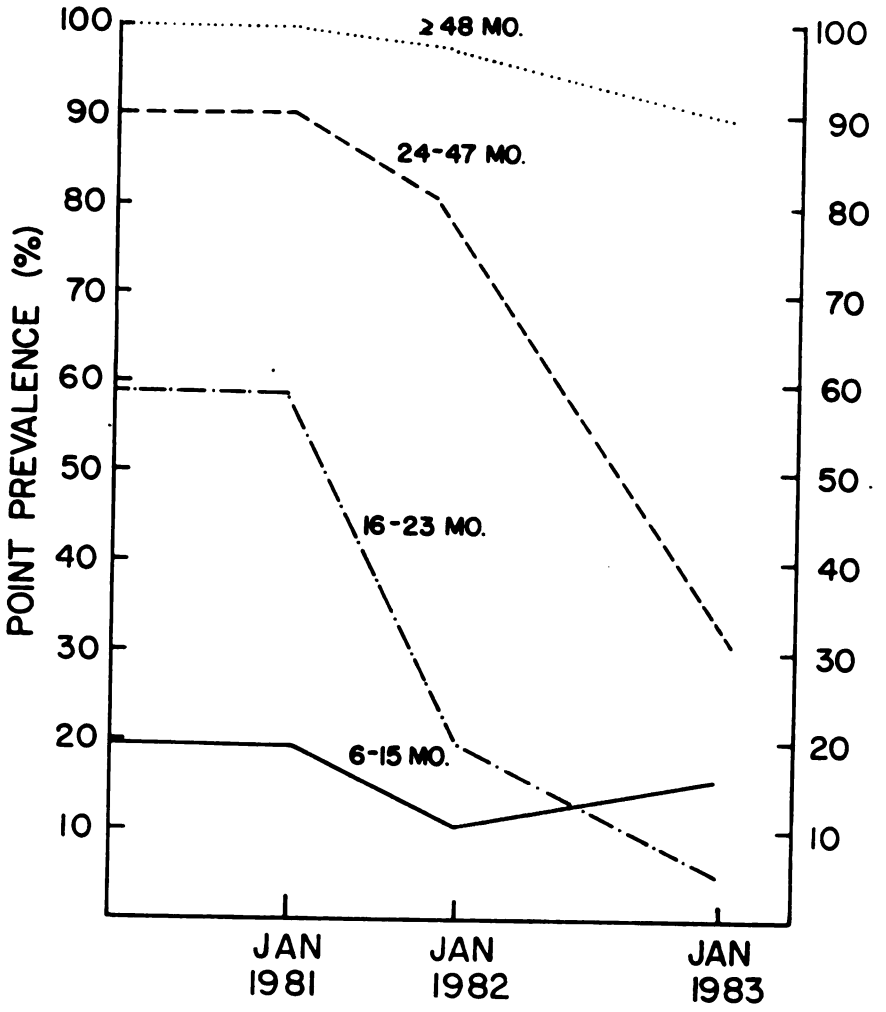

Figure 1.2: Age specific point prevalence of AGID reactors in 4 age groups.

TABLE 1.1

Point prevalence of AGID reactors in 4 age groups.

	JAN 1981		JAN 1982		JAN 1983	
Age (Mo.)	No. Tested	% Pos.	No. Tested	% Pos.	No. Tested	% Pos.
6-15	32	18.8*	40	12.5	44	16.2
16-23	19	57.9	39	20.5	30	13.3
24-47	56	89.3	74	81.0	82	32.0
48	21	100.0	40	97.5	42	90.2
Total	128	68.8**	193	58.0	193	37.4

* = % positive for an age group; ** = % positive of entire herd. Increase from 128 to 193 coicides with the retention of all seronegative heifers and extends over 2 calving seasons.

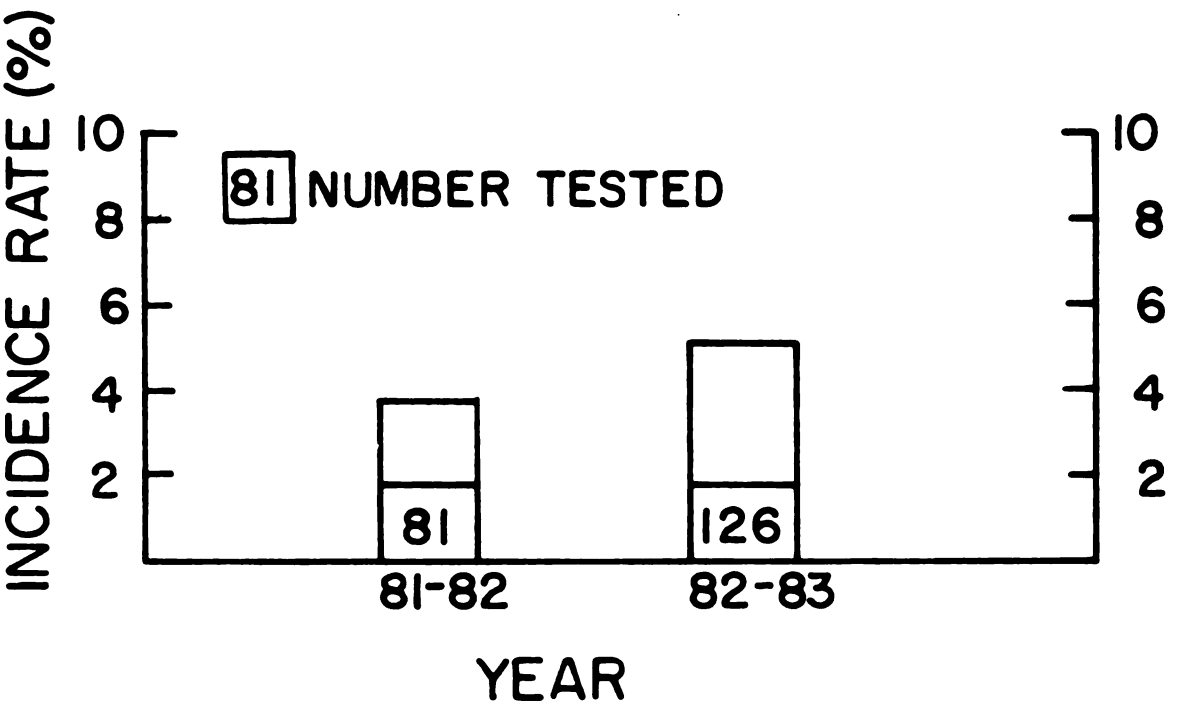

Figure 1.3: Incidence rate of AGID reactors at age ≥ 6 months.

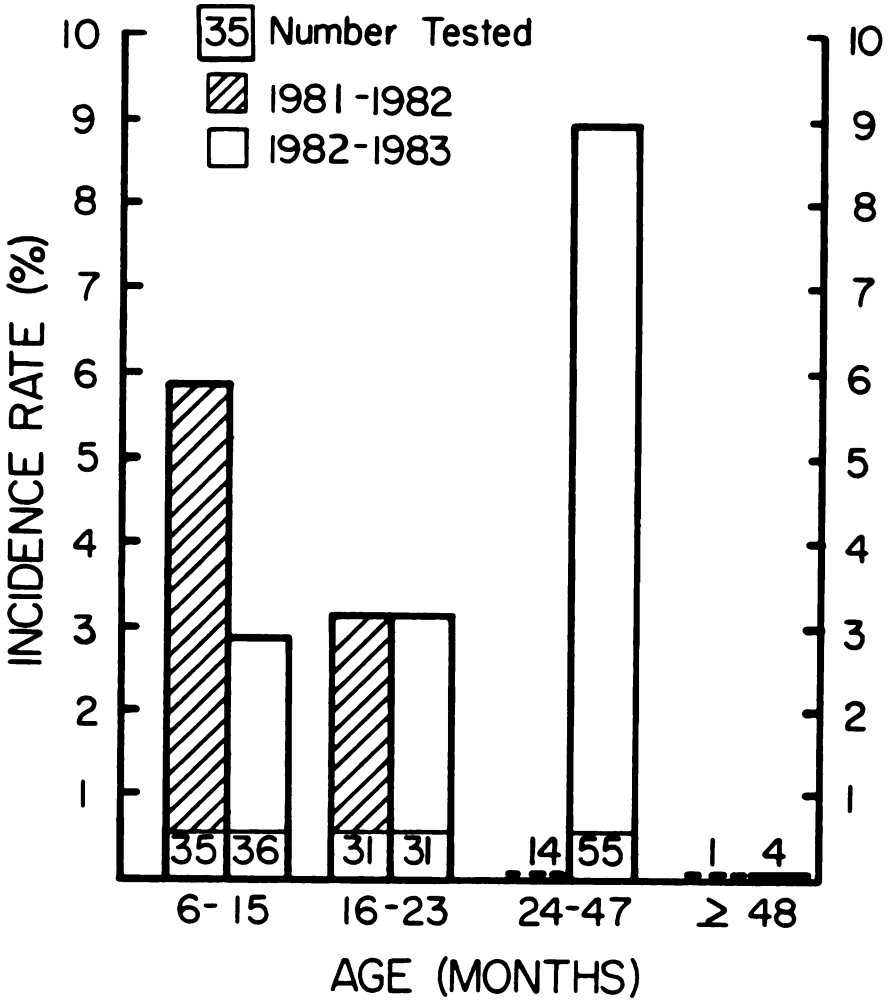

Figure 1.4: Comparison of the incidence rate of AGID reactors for 4 age groups.

seroconversions in the herd in 1982 were in that 24-47 month group. Adding BLV negative cows in this age group to the milking herd resulted in closer physical contact with the BLV positive cows, even though negative and positive cows were separated. Increasing the amount of physical contact has been shown to increase the incidence rate whereas less physical contact results in decreased incidence rates (Wilesmith et al., 1980; Thurmond et al. 1983).

There are several characteristics of this herd, in contrast to privately owned herds, which increased the number of variables that could have affected the changes in PPR and IR. The risks of transmission were higher because of the multiple purposes for which the herd was used. The number of personnel was larger, and the personnel's turnover rate was very high. It was necessary that constant cognizance be made of the importance of following the guidelines. These factors would favor slower decreases in PPR and increases in IR in this study. It would be exceptional for these problems to exist in a commercial dairy.

A factor which would favor faster decreases in PPR and IR in any herd would be culling of cows at a young age. This is especially true for BLV since the PPR of BLV is higher in older cows. Older cows would not be available for transmission of the virus if the age at culling is

young. We had hypothesized that if there was a significant difference between the average age of lactating cows in this herd when compared to commercial dairy herds, the decreases in PPR and IR obtained in this herd in the 3-year period would not be comparable to what one would expect in commercial dairies. The average age of lactating cows in this herd, however, was comparable to commercial herds in Michigan (49.5 months and 50.5 $\pm$ 0.5 months for 196,000 cows, respectively). Thus, age offered no apparent bias in these results. Because of this observation, the results obtained in this herd may, in fact, be applicable to commercial herds.

Another factor favoring the practical applicability and success of these control measures is the PPR for the general cattle population. The 95% PPR in the MSU dairy is greater than 3-fold the PPR (30%) for the State of Michigan (Kaneene, personal communication, 1983). A similar state PPR (47.8%) was found in dairy cows surveyed in Florida (Burridge, 1982b). Many commercial dairies, then, may actually start control and eradication programs at the PPR at which this study was virtually terminated and at a PPR (34%) that was attained over a 3-year period. Hence, it may take those herds less than 3 years to attain their goals.

Since there was a further decrease of 3% in the PPR

in this study 10 months after strict separation of the cows was discontinued, further decreases also may be expected in commercial herds. The success of this modification could be dictated by the PPR in the herd at the time of discontinuation as well as the ability to adhere to the remaining control measures. A low PPR would decrease the number of cows available for transmission of BLV.

A lactating cow's BLV antibody status was not used as a determinant for culling as were diseases like mastitis, infertility and lameness. This contrasted control programs in other countries (Mammerickx, 1978; Mammerickx, 1982; Yoshikawa, 1982) in which slaughter of BLV positive animals was performed because of a BLV positive serotest. Thus, fewer economic losses due to slaughter were incurred in this study. Slaughter is not an economically viable option in the United States, because indemnity is not offered.

CONCLUSIONS

The results of this study correlate with those of other researchers (Mammerickx et al., 1978, Yoshikawa et al., 1982, Ruppanner et al., 1983, Kaja et al., 1984). Control of and possibly eradication of BLV apparently is quite feasible. The rapidity with which control is

gained depends on the prevalence of the infection within the herd, the degree to which sanitary measures are adhered to, and the willingness of owners to separate or to cull eligible seropositive animals. This herd was used for teaching, research and public visitation, and the labor force was constantly changed. None of the animals were culled strictly because of a positive serotest.

Unlike privately owned herds, there were greater opportunities for spreading BLV to susceptible animals. Favorable results were obtained in spite of the greater opportunities to infect the susceptible herd members.

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PART TWO

BOVINE LEUKEMIA VIRUS: THE ROLE OF PHYSICAL SEPARATION IN CONTROLLING THE SPREAD OF THE VIRUS

ABSTRACT

A study was conducted using the Michigan State University dairy herd to determine the importance of the role of physical separation in controlling the spread of bovine leukemia virus (BLV). Control measures that required physical separation of BLV seropositive animals from BLV seronegative animals and that required numerous sanitary measures were employed from November 1979 through December 1982. Physical separation of the animals was discontinued in January 1983. The animals then were housed based on age, weight, level of production, and lactation status. The sanitary measures employed prior to 1983 were not changed. The agar gel immunodiffusion (AGID) test was used to detect antibodies to BLV. The results of the AGID test were compared for 1982 (physical separation plus sanitation) and 1983 (sanitation only). Herd and age specific point prevalence rates (PPR) and herd and age specific incidence rates (IR) were analyzed for the "whole herd" (included animals purchased in 1983) and for the "partial herd" (included only those animals born at the dairy). The data were analyzed using the chi square test at a probability level of 0.05. There

was no significant difference in the increase in the PPR for the "whole herd" (37.8% to 46.0% BLV seropositive). There was a significant difference in the increase in the PPR for the "partial herd" (37.8% to 50.4% BLV seropositive). There was a significant difference in the increase in the yearly PPRs for the 16-23 month age group and the yearly PPRs for the 24-47 month age group for the "whole herd", but there was no signficant difference in the yearly PPRs for any age group in the "partial herd". Though there was no significant difference in the herd IRs (pooled population at risk) for 1982 versus 1983, the yearly age specific IRs (age specific population at risk) for the 16-23 and 24-47 month age groups were significantly different. The results showed that physical separation was important for controlling the spread of BLV in a herd with a high prevalence of BLV antibodies. Seronegative animals between the ages of 16 months and 47 months were at a higher risk of contracting BLV under the conventional housing conditions used in this study.

INTRODUCTION

Several studies have employed procedures designed to control the spread of bovine leukemia virus (Mammerickx et al., 1978, Ferdinand et al., 1979, Ruppanner et al., 1983, Kaja et al., 1984, Johnson et al., unpubl.). The methods of control in those studies can be categorized

into 2 major groups, those being either physical separation or sanitation. Because there was an attempt to block every possible route of transmission of BLV in those studies, all the control measures involving both separation and sanitation were employed simultaneously. None of the control measures were studied as an isolated variable to determine the relative importance of individual variables.

Because of the limited availability of housing on most farms, attempting to physically separate BLV negative from BLV positive cows would be impractical, uneconomical, and laborious. Separating the positive from the negative animals could lead to several managerial problems. Adult cattle on most farms are separated usually on the basis of their milk production, pregnancy status, and lactation status. Heifers and calves are usually separated on the basis of body weight and age. If one further attempts to separate each of these groups based upon the animal's BLV status, the number of different groups of animals could potentially double. The difficulty of managing an operation of this type probably would parallel the number of separate units for the animals.

From a practical standpoint, it becomes necessary to determine the importance of the role of physical separation in controlling the spread of BLV. If separation of BLV positive from BLV negative cows is proven to be unimportant in

controlling the spread of BLV, the recommendations for the control of BLV listed in previous studies would be easier to employ and thus would be more appealing to livestock owners.

OBJECTIVE

To determine the role of physical separation in controlling the spread of bovine leukemia virus.

MATERIALS AND METHODS

With few exceptions, the materials and methods of this study (study 2) did not differ from those employed in a previous study (study 1) by Johnson et al., unpublished.

Housing, Separation and Sanitation

Beginning in January 1983, the strict separation of BLV positive from BLV negative cows which was practiced from November 1979 through December 1982, was discontinued. The cows were housed based on the age, weight, lactation status, and pregnancy status as housing had been done prior to the start of study 1 in November 1979. The animal's BLV antibody status was not regarded when its housing assignment was made. However, all sanitary measures employed between November 1979 and December 1982 (Part 1) were also employed in part 2.

Purchase of New Cows

Twenty-six heifers between the ages of 2-21 days were purchased in July and August of 1982 for use in a research project.The BLV status of these heifers was not determined prior to their purchase. An experimental procedure involving control of exposure of these calves to specific amounts of daylight hours required that the calves be separated from the remainder of the dairy herd. This separation was terminated in January 1983, at which time they became embryo recipients and were mixed with heifers that were born and raised at the dairy.

Testing Schedule

The entire herd was tested for BLV antibody during October 1983 and early January 1984. The procedures for collection and storage of blood and the procedures for the serological test remained the same as described in a previous study (Johnson et al., unpubl., Miller and Van Der Maaten, 1976).

Definition of Terms

Whole herd point prevalence rate (PPR). PPR calculated using all animals in the January 1984 herd that were at least 6 months of age. This included animals born on the farm as well as purchased animals.

Partial herd point prevalence rate, PPR calculated using

only that portion of the January 1984 herd that was born on the farm and was at least 6 months of age. This excluded any purchased animals.

Age specific point prevalence rate. PPR calculated for each of 4 age groups (6-15, 16-23, 24-47, and $\geq$ 48 months of age).

Annual herd incidence rate (IR) - defined as the number of new animals with a positive BLV serotest divided by the population at risk in that year. Annual age specific incidence rate - Annual incidence rate calculated for each of the 4 aforementioned age groups.

Statistical Analysis of Results

The working hypothesis for both the prevalence rates and incidence rates was that there would be no significant differences between increases in the herd and age-specific prevalence and incidence rates in the absence of physical separation of BLV positive from BLV negative animals.

A whole-herd point prevalence rate was used to determine the magnitude of BLV infection as determined by the presence or absence of BLV antibody titers in the entire herd. In addition to the whole-herd PPR, a partial-herd PPR was calculated. The animals used to compute the partial-herd PPR was determined by calculating the difference between the number of animals in the entire herd in

January 1984 and the number of animals purchased. Whole herd age specific point prevalence rates and partial-herd age-specific point prevalence rates were calculated for age groups of 6-15 months, 16-23 months, 24-47 months, and animals greater than or equal to 48 months. An examination for significant differences between the whole-herd point prevalence rate of December 1982 and December 1983, the partial-herd point prevalence rate of December 1982 and December 1983, and the age-specific point prevalence rates of the whole herd and the partial herd for both years was performed using the chi-square test of independence at a probability level of $p \leq 0.05$.

An incidence rate was used to measure the risk of an animal's developing detectable BLV antibodies in a stated time period. The incidence rates for 1982 (sanitation plus physical separation) and 1983 (sanitation only) were compared. The incidence rate for 1982 was calculated using the eligible population from the entire herd. The incidence rate of 1983 was calculated using only the eligible population from the partial herd. The purchased animals could not be used in the calculation of the incidence rate of 1983 since there were no baseline serological data for them. Age-specific incidence rates were calculated for age groups of 6-15 months, 16-23 months, 24-47 months and animals greater than or equal to 48 months. An examination for

significant differences between these incidence rates was calculated using the chi-square test of independence at a probability level of 0.05.

RESULTS

Point Prevalence Rate (PPR)

The whole herd point prevalence rate for 1983 was determined using the entire herd of 189 animals. There was no significant difference ($p \geq 0.05$) between the whole-herd PPR of 1982 and 1983 (Fig. 2.1). There also was no significant difference between the 6-15 month age group of 1982 and 1983 and the 48 month and older age groups of 1982 and 1983 (Fig. 2.1). There was a marked significant difference ($p \geq 0.05$) between the 16-23 month age groups and the 24-47 month age groups of 1982 and 1983 (Fig. 2.1).

The partial-herd point prevalence rate was determined using only 141 of the 189 animals used to calculate the whole-herd point prevalence rate. There was a significant difference ($p \leq 0.05$) between the partial-herd PPR of 1982 and 1983 (Fig. 2.2). However, there was no significant difference ($p \geq 0.05$) between any of the partial-herd age-specific PPRs of 1982 and 1983 (Fig. 2.2).

Incidence Rates (IR)

Eighteen of 89 BLV negative animals seroconverted

between January 1983 and December 1983. There was no significant difference ($p \geq 0.05$) between the herd IRs (pooled population at risk) of 1982 and 1983 (Fig. 2.3). An examination for differences between age-specific IRs (age specific population at risk) for 1982 and 1983 revealed significant differences ($p \leq 0.05$) with the 16-23 month and the 24-47 month age groups only (Fig. 2.4). Neither a Chi-square test nor a Fisher's exact test could be performed on the 48 month age group because of the small number of animals.

DISCUSSION

Point Prevalence Rate (PPR)

There are several possible factors which may have contributed to the marked significant differences between the whole-herd age-specific PPR of the 16-23 and 24-47 month age groups. The 26 heifers purchased during July and August of 1982 were between 17 and 18 months of age at the time of the January 1984 testing and were categorized in the 16-23 month age group. They comprised 47.27% of the 55 animals in that age group. Their BLV status was unknown at the time of their being purchased and was unknown at the time that they were mixed with BLV negative heifers of their ages. The sanitary measures used in a previous study (Johnson et al., Prev. Vet. Med., in press,

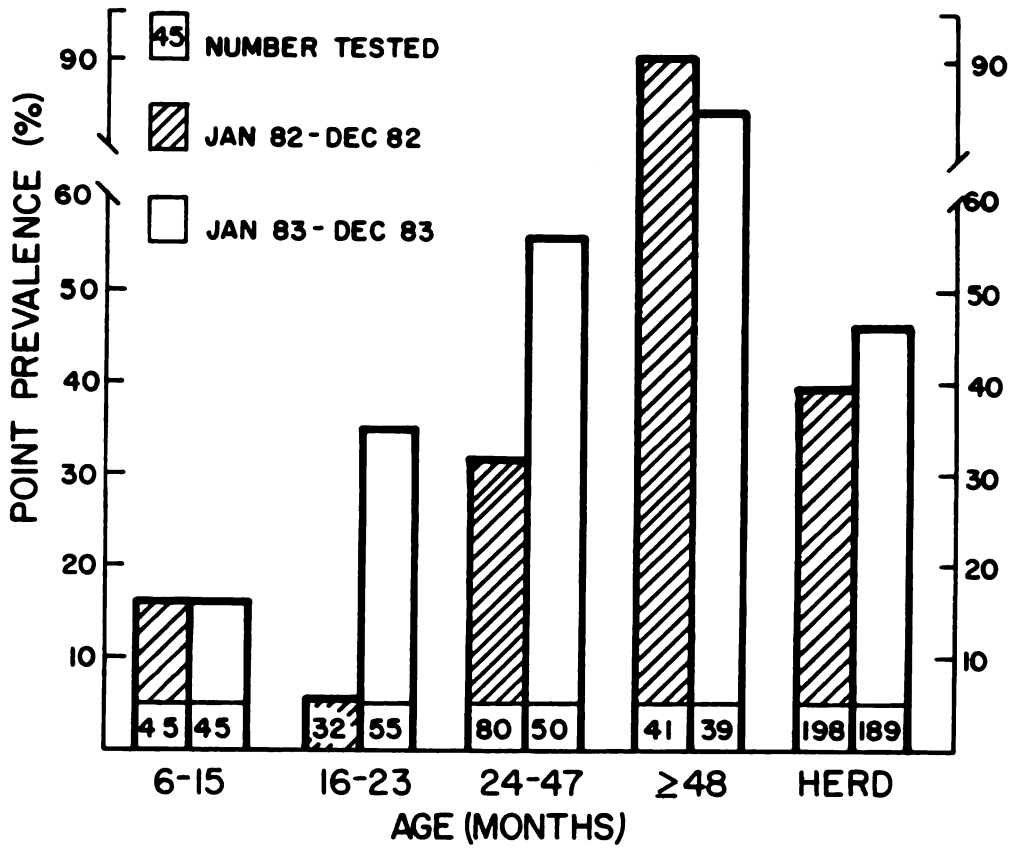

Figure 2.1. Whole-herd age specific point prevalence rates of AGID reactors > 6 months of age. * Purchased animals included for 1983.

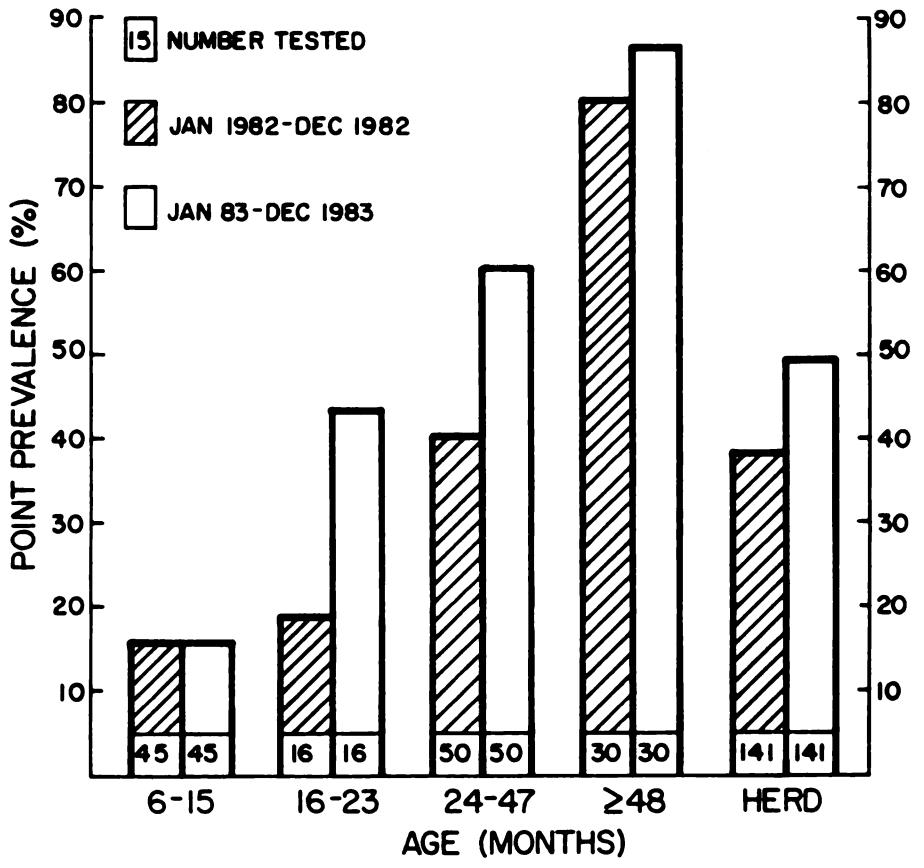

Figure 2.2. Partial-herd age specific point prevalence rates of AGID reactors > 6 months of age. *Purchased animals excluded for 1983.

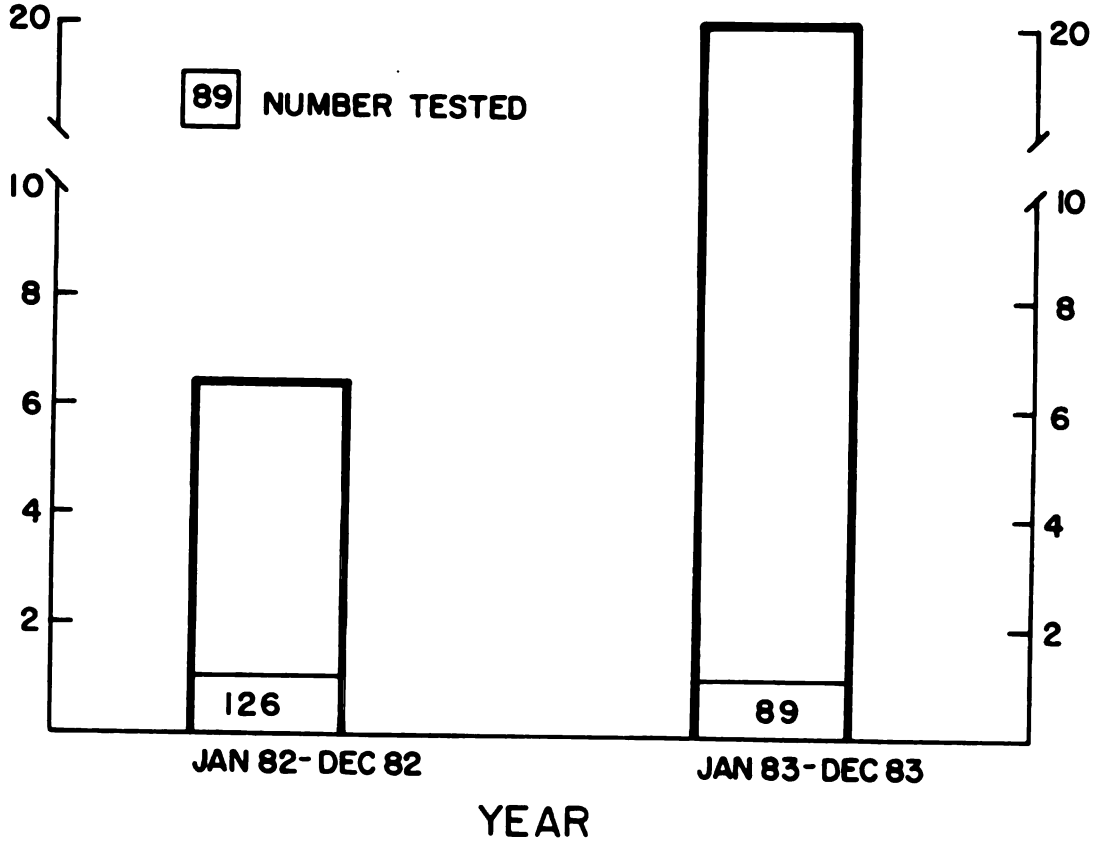

Figure 2.3. Annual herd incidence rate of AGID reactors at age > 6 months of age. *Purchased animals excluded for 1983.

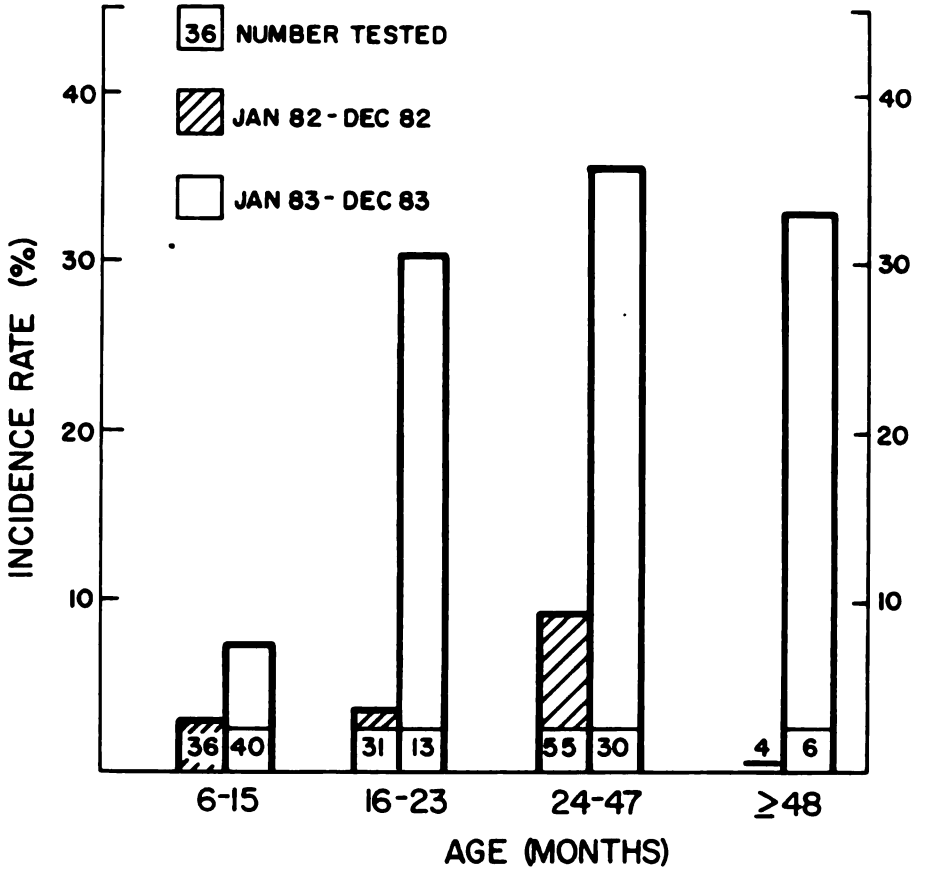

Figure 2.4. Comparison of annual incidence rates of AGID reactors for 4 age groups. *Purchased animals excluded for 1983. Separation of AGID (+) and AGID (-) animals discontinued in January 1983.

1985) for the development of a herd-based control strategy for BLV were not utilized on these heifers since they were not expected to remain with the herd. At the time of their first BLV serotest in early January 1984, 15 of the 26 (57.69%) purchased animals were BLV seropositive whereas only 2 of 29 (7.69%) of the 16-23 month old farm-raised animals were BLV seropositive simultaneously, and only 8 of 39 (20.5%) of the farm-raised animals were BLV seropositive the previous year. Thus, the addition of BLV positive purchased animals rather than transmission of BLV to susceptible animals may have been the major cause of the significant difference between the PPR in the 16-23 month age group.

No purchases were added to the 24-47 month age group. Unlike the 16-23 month group, the significant difference between the PPR can be accounted for only by their having contracted the virus via breaks in sanitation. It is well accepted that the transfer of viral-infected lymphocytes rather than mere physical contact is important for transmission of BLV to occur. Since BLV positive and negative cows were mixed, the intermingling could have increased the probability of transmission via accidental breaks in sanitary guidelines. Thus, the significant difference in PPR of this age group may not have been due only to the fact that positive and negative cows were housed together

but more importantly it may have been due to the increased transmission that may have occurred because they were housed together.

The lack of significant differences between the 6-15 and 48 month and older groups was consistent with the results from the physical separation-plus sanitation studies. The PPR in study 1 ranged from 12.5% to 18% for the 6-15 month group and 90.0% to 100.0% for the 48 month and older age group. A high percentage of young animals have consistently been shown to be BLV seronegative precolostrally and after BLV colostral antibodies have disappeared (Van Der Maaten et al., 1981, Thurmond et al., 1982, Thurmond et al., 1983, Jacobsen et al., 1983 Johnson et al., unpubl.). As the animals get older they tend to become seropositive unless there is an effort to prevent their contracting the virus.

It was necessary to calculate a partial-herd PPR to remove the possible bias caused by the addition of purchased cows whose BLV status was not known. Thus, there were 141 cows common to both yearly studies. The absence of a significant difference between the PPRs of those age specific groups indicates that even though there was intermingling of these cows between January 1983 and January 1984, mere physical contact for 12 months did not significantly increase the point prevalence of either age group.

INCIDENCE RATES

In spite of a 30.0% decrease (126 vs 89) in the total number of cows tested for seroconversion between 1982 (sanitation plus physical separation) and 1983 (sanitation without physical separation), there was a 3-fold increase in the herd's incidence rate (Fig. 2.3). Fifteen of the 18 seroconversions (83.33%) occurred in the 16-23 (22.22%) and 24-47 (61.11%) month age groups (Fig. 2.4). The marked significant differences between the incidence rates in those two age groups also may have the same explanation as the differences seen in the whole-herd age-specific PPRs and partial-herd age-specific PPRs i.e. physical contact allowed easy accidental transmission of the virus.

The primary aim of this study was to determine the significance of the role of the single variable of physical separation upon the control of the spread of bovine leukemia virus. The reasons for the importance of determining whether it is necessary to separate BLV positive from BLV negative animals were discussed. The 16-23, 24-47, and 48 months and older age groups were most useful for drawing conclusions from this study. Because there was no significant difference between the partial-herd age-specific PPRs for either of these 3 groups, it was not shown using the point prevalence rate that it is necessary to isolate BLV positive from BLV negative animals as long as proper sanitary

measures were employed. On the contrary, utilization of the incidence rate produces the exact opposite results because a significant difference was shown for the 2 younger of the 3 age groups used in the analysis, and the incidence rate was markedly increased for both groups. Even though there was no significant difference shown by the 6-15 month age group for either the whole-herd age specific PPRs, partial-herd age specific PPRs, and age specific incidence rate, utilization of this group to draw conclusions was not helpful since these animals have been shown consistently to have a low PPR and IR. Also, they were never exposed to the BLV positive cows in the milking herd, and they were handled much less frequently for inseminations, vaccinations, etc.

The results of this study must be interpreted in light of the "potential dynamics of transmission" in this herd (potential dynamics which are high) versus privately owned herds. The concepts of herd immunity (Schwabe et al., 1977) must be considered before making any recommendations as to how these 4 age cohorts should be housed. Cohorts with a high PPR probably will require physical separation. There will be a sufficient number of BLV positive animals in such cohorts to increase the probability that a susceptible animal will become infected easily. On the other hand, cohorts in which the PPR is low may not warrant physical

separation. These conclusions are based on the assumption that strict sanitary guidelines will be employed to prevent the spread of BLV infected lymphocytes. If strict guidelines for sanitation are adhered to by privately owned herds, reasonable control of this virus could possibly be gained without employing physical separation in spite of the results demonstrated by this study.

The results of this study show that physical separation of BLV positive from BLV negative animals is important for limiting the spread of BLV. The animals 16 months to 47 months of age were at a higher risk of contracting BLV. Farmers with managerial conditions similar to those in this study who wish to export cattle should consider either marketing them prior to this age, or become especially careful to prevent these animals from becoming infected if they are mixed with BLV positive animals, or maintain separate facilities for the seronegative animals.

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PART THREE

BOVINE LEUKEMIA VIRUS: DURATION OF BLV COLOSTRAL ANTIBODIES IN DAIRY CALVES IN MICHIGAN

ABSTRACT

A study was conducted to determine the duration of colostral antibodies to bovine leukemia virus (BLV) in Michigan diary calves. Sera of pregnant dams from 4 different dairy farms and sera of the calves of these dams were analyzed for BLV antibodies using the agar gel immunodiffusion (AGID) test. Precolostral serum samples were collected from the female calves of known BLV serologically positive dams. Postcolostral serum samples of the same calves were collected on day 2 and biweekly until 2 consecutively negative AGID tests performed 4 weeks apart were obtained. Subsequently, the biweekly serum samples from each calf were analyzed quantitatively for BLV antibodies with the AGID test. End-point titers were determined using phosphate buffered saline to make two-fold dilutions. A logarithmic transformation of the inverse of the end-point titer was used to determine the regression line of antibody decay for each calf. An estimated weighted regression analysis was used to determine the least-squares regression line for 27 of the 38 calves. The duration of colostral antibodies was calculated as 71 days using the prediction equation. The range of the

duration of colostral antibodies was 14 days to 147 days. The half-life of the antibodies was 36.05 days. Factors affecting the duration of BLV colostral antibodies and the practical applicability of this study are discussed.

INTRODUCTION

There is much interest in the control of bovine leukemia virus (BLV) infection in the United States as well as in European countries (Bause et al., 1978, Mammerickx et al., 1978; Ruppanner et al., 1983; Kaja et al., 1984). As important feature of an efficient disease control program is to prevent the further spread of the disease by the early detection and removal of infected animals. Calves in BLV control programs are not tested initially until approximately 6 months of age (Straub, 1978, Johnson et al. unpubl.). This delay in testing for BLV is unavoidable because the most commonly used diagnostic test for BLV is a serologic test for BLV antibody (Mammerickx et al., 1978, Straub, 1978). Presently there is no diagnostic test for discriminating between passive colostral BLV antibodies and active BLV antibodies. A procedure which would help to discriminate between colostral and active antibody would increase the efficiency of a BLV control program.

Vaccination may become a viable approach to the

control of BLV (Miller and Van Der Maaten, 1978, Ferrer, 1980, Miller et al., 1983, Parfanovich et al., 1983). An important objective of a vaccination program is early immunization of a high percentage of animals. Since the efficacy of a vaccine is influenced by passive antibodies (Uhr and Baumann, 1961, Brar et al., 1978), quantitative information about the concentration and duration of BLV colostral antibodies would aid in the development of vaccination strategies.

OBJECTIVE

The objective of this study was to determine the duration of colostral antibodies to BLV in calves as measured by the agar gel immunodifussion test and to estimate the normal limits of this duration. An estimate will be given for the age at which dairy calves in Michigan can be expected to have negligible amounts of BLV colostral antibodies.

MATERIALS AND METHODS

Solicitation of Client Participation

The clinic records and records from the animal health diagnostic laboratory at Michigan State University were reviewed for all cases of bovine lymphosarcoma diagnosed during 1978 through 1983. Because of the possibility of

there being a higher BLV prevalence rate in those herds as well as more willingness to participate in studies which could lead to better control of the disease, these clients were requested to participate in the study. Four dairy farmers participated. Sera were collected from 38 calves over a 7-month time period.

Sampling Instructions and Sampling Schedule

Sampling Instructions.

During a visit to the farm each farmer was given specific instructions for jugular venipuncture of the calves and coccygeal venipuncture of the dams. Materials for recording the date on which blood samples were to be collected and on which the test results were to be recorded were dispensed to the farmer.

Sampling Schedule.

Blood collected from each cow during the farm visit was screened for antibodies to BLV. A record was made of the expected calving date for all cows that had antibodies to BLV. Another blood sample was taken from the dam by the farmer on the day of parturition. A precolostral blood sample was taken by the farmer from each female calf on its first day of birth, on its second day of birth, and biweekly until two consectively negative serotests taken 4 weeks apart were obtained. Each blood sample was allowed to clot at a room temperature which varied slightly among the farms. To prevent

hemolysis,the client was instructed to remove the retracted clot using a sterile wood applicator stick and to refrigerate the serum at 2°C. After several samples had accumulated, they were mailed to veterinary medical teaching hospital for analysis. The results of the tests were reported to the farmer immediately.

Laboratory Tests

Qualitative analysis for BLV antibodies: Sera collected from the dams and calves were analyzed for antibodies to the BLV glycoprotein-51 antigen using the commercial agar gel immunodiffusion test.[a] If it was unknown by the client as to whether a calf received colostrum prior to collection of the first blood sample, the serum was also analyzed for immunoglobulins using the zinc sulfate turbidity test (McEwan, 1970). If the immunoglobulin concentration was less than 600 mg/dl and the AGID test was positive, the calf was discontinued from the study since the BLV antibody causing the positive AGID test was probably due to an in utero initiated active antibody response. If the immunoglobulin concentration was normal ($\geq$ 600 mg/dl) and the AGID test was positive, the calf was continued in the study because the BLV antibody causing the positive AGID test was probably due to passive transfer of BLV antibodies in the dam's colostrum.

Quantitative analysis for BLV colostral antibodies:

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[a]Leukassay B, Pitman-Moore, Washington Crossing, NJ

A quantitative analysis for BLV antibodies was performed also using the AGID test and the Leukassay B Kit. An end-point titer for each AGID positive sample was determined. Two-fold dilutions were performed with phosphate buffered saline. End-point titers from 38 calves were analyzed for colostral antibodies.

STATISTICAL ANALYSIS AND RESULTS

The working hypothesis was that the mean duration of BLV colostral antibodies is less than the traditionally accepted 6-month duration. To determine the individual regression line for each calf, a logarithmic transformation of the inverse of the end-point titer was performed. The equation of the regression line for any one calf can be expressed as:

$$\log_{10} Y = a + bX + e \qquad \text{(equation 1)}$$

where Y is the logarithm to the base 10 of the inverse of the endpoint titer

a is the y-axis intercept
b is the slope of the regression line
X is the age in days when the end-point titer was observed
e is the error term

Eleven (28.94%) of the 38 calves were ineligible for the statistical analysis because at least 3 positive observations were required to estimate the variance (Neter and Wasserman, 1974; Schfler, 1980) (Fig.3.1 and Fig.3.2).

Estimates for a and b were obtained for each calf. These estimates were pooled and weighted and used to write the combined regression line (Swamy, 1971; Neter and Wasserman, 1974; Thurmond et al., 1982; Carter, personal communication, 1984). The prediction equation for these 27 calves is:

$$\text{Log}_{10} Y = .59181 - .00835(X) \quad \text{(equation 2)}$$

If we assume that protection is gone when the end-point titer is unity, then the above equation solved for X gives:

$$X = 70.88 \text{ days}$$

The half life of the antibodies can be estimated using an equation given by Thurmond et al., 1982:

$$T^{1/2} = \log_{10}(.5)/b \quad \text{(equation 3)}$$
$$= 36 \text{ days.}$$

The number of positive AGID test for any one of the 27 calves ranged from 3 to 7 using the qualitative analysis. The weakest dilution at which a positive AGID test was still detectable was 1:64 using the quantitative analysis. The duration of colostral antibodies to BLV for the 27 calves with at least 3 positive observations was between 42 and 133 days (Figs. 3.1 and 3.2). The duration of antibodies for the remaining 11 calves having less than 3 observations was between 14 and 27 days. The arithmetic mean and median duration for the 27 calves were 60 days

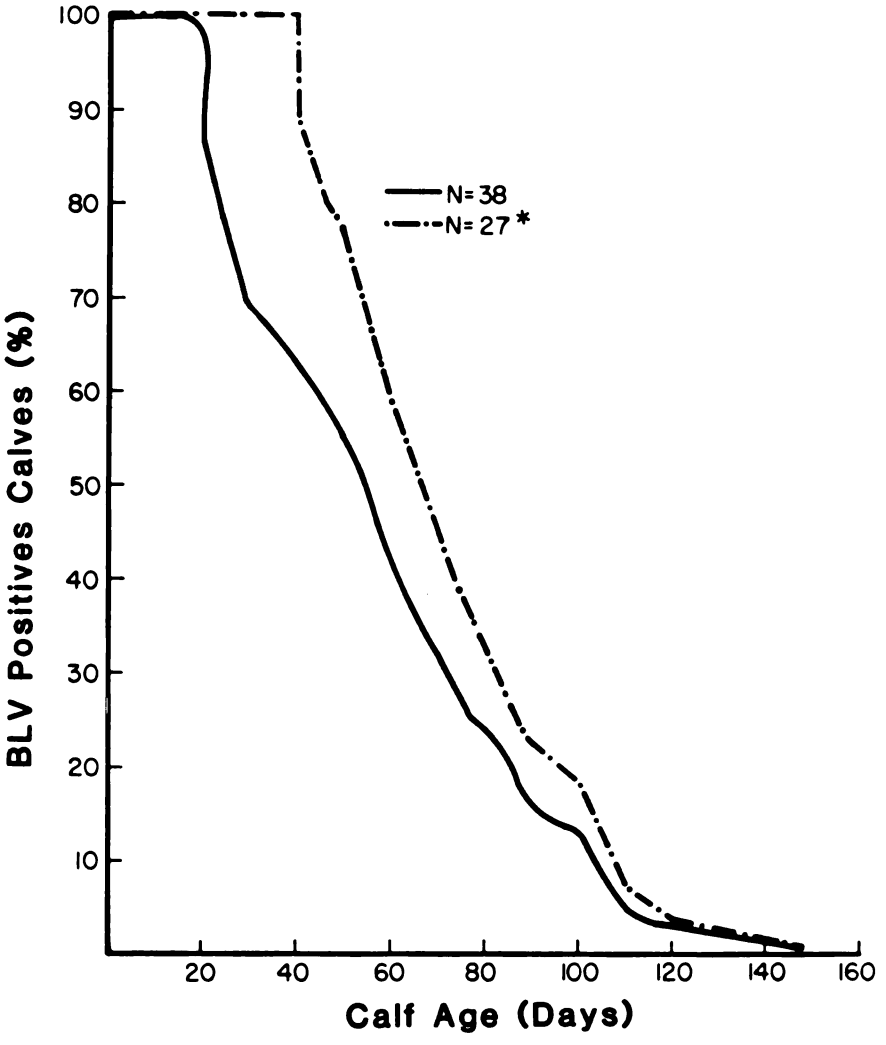

Figure 3.1. Percent of 38 calves with BLV colostral antibodies as a function of age. *N = 27 includes only those calves with at least 3 AGID (+) observations.

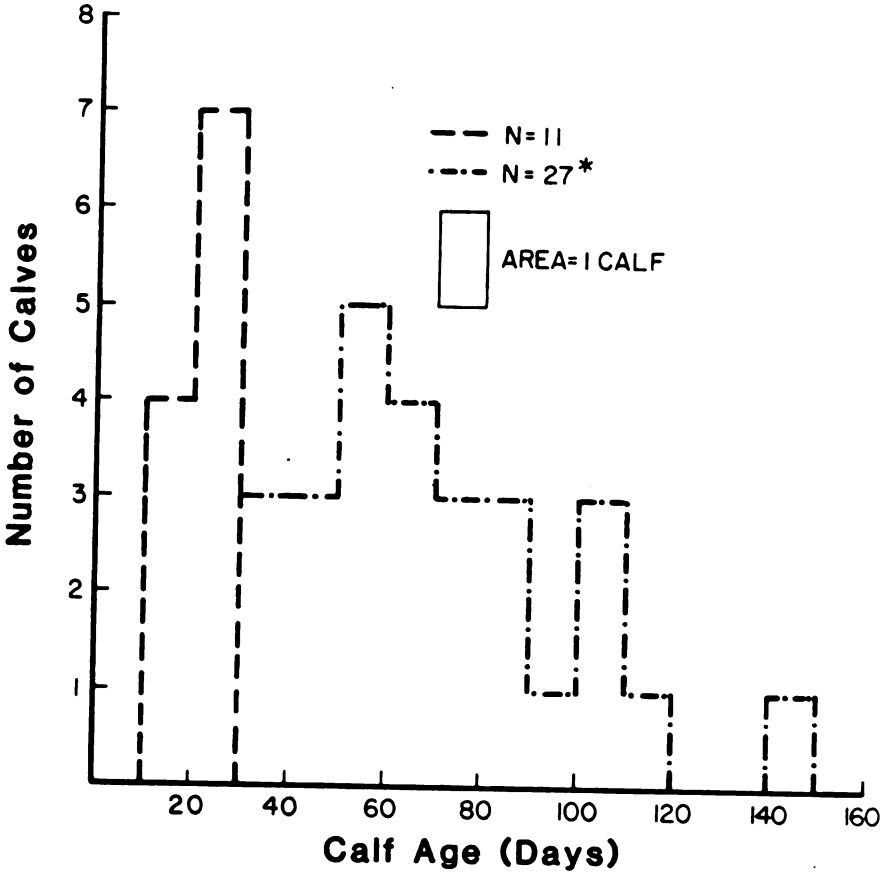

Figure 3.2. Histogram of 38 calves with BLV colostral antibodies versus the age at the first negative AGID test. *N = 27 includes only those calves with at least 3 AGID (+) observations.

and 53 days, respectively. The 95% and 99% confidence intervals were 70.90 days and 74.40 days, respectively. The half-life of the antibodies was 36.05 days. The small number of data points on the scattergram prohibited a useful graphic representation of the prediction line.

DISCUSSION

The primary immunoglobulin absorbed by the neonatal calf after consumption of colostrum is IgG1 (Brandon et al., 1977). These immunoglobulins originate in maternal circulation. They are transferred into the mammary gland approximately 5 weeks prior to parturition (Brandon et al., 1977). The agar gel immunodiffusion test in which the glycoprotein antigen (gp-51) is used measures IgG1 (Matthaeus et al., 1978). Thus, the decay of colostral antibodies to BLV essentially is the decay of IgG1, and vice versa.

In 2 other studies, the longest duration of BLV colostral antibodies were 154 days (Fischer and Keyserlingk-Eberius, 1980) and 187 days (Thurmond et al., 1932). There are intrinsic and extrinsic factors for these discrepancies in the duration. Extrinsic factors which may determine the level of specific antibodies absorbed by the neonatal calf are the geographical variations in the prevalence of antibodies to BLV virus, the herd prevalence of

antibodies to BLV virus, and the quantity and quality of antibodies produced by individual dams. The intrinsic factors which may cause this discrepancy are the quantity and quality of antibodies absorbed by the calf, the presense of enteric disease which may result in malabsorption of colostrum, and the lack of standarization of the AGID test.

The importance of the individual intrinsic factors from the greatest to the least importance probably follows the pattern listed in the aforementioned paragraph. Geographical and herd variations in the prevalence of BLV have been documented (Burridge, 1981, Kaneene, personal comm., 1984). The quantity and quality of antibody produced by an individual dam may vary with her degree of exposure to antigens as well as unique physiological variations in the immune response. Though all of the dams in this study had positive AGID tests 4-8 weeks prepartum, 5(18.15) had developed a weak positive test and 9 others (33.0%) had developed negative tests on the day of parturition. The low periparturient level of circulating maternal antibody due to transfer of much of this antibody into the colostrum (Klaus et al., 1969, Penhale and Christie, 1969, Husband et al., 1972) could explain the weak positive or negative serotests of the dams. The calves from dams with a negative serotest on the day of parturition

developed their first negative serotest by day 56 whereas those calves from dams with a positive serotest required as long as 147 days before the first negative serotest developed (Table 3.1). The BLV antibody titers of the sera collected from the dams on day 1 could not be analyzed quantitatively due to insufficient serum. Thus, a correlation between maternal and calf serum concentrations of BLV antibody could not be made. However, the evidence above indicates that calves of some dams may received higher quantities of BLV antibodies since there was such a wide range in the durations. This evidence also indicates that serotesting animals within 6 weeks of parturition may result in numerous false negatives. A final extrinsic factor is that some dams may leak colostrum from the udder prior to parturition and thus deny the calf colostrum with a high antibody concentration.

Regarding intrinsic factors, it is unlikely that malabsorption of antibodies plays a major role in shortening the duration of antibody decay if it is assumed that the quality and quantity of colostrum ingested is normal. Infectious agents, be they viral, bacterial or protozoal, are the primary causes of acute-onset, malabsorptive enteric disease in neonatal calves because of the altered secretory processes or destruction of the intestinal mucosa. Their role in inhibiting absorption of colostral antibodies

is probably minor unless intestinal invasion occurs prior to the period in which the bulk of antibodies are absorbed i.e. the incubation period of the infectious agent is shorter than gut closure time. The results of the AGID test are measured on a discrete scale (positive, weak positive, negative). This discrete measurement also may explain the discrepancy in the durations because test re-results could vary among and within the institutions conducting the study.

The date for establishing the duration of colostral antibodies was selected by using the date of development of the first negative AGID test. Since the blood samples were collected at 2 week intervals, there was a 2-week time span between the last positive and the first negative test. Thus, the duration of antibodies for each calf could be a maximum of 13 days shorter than the duration graphed (Figs. 3.1 & 3.2). In other words, the X coordinate of the points forming the lines of Figure 3.1 and the bars on the histogram could be shifted 13 days closer to the Y axis. The flexible shift-to-the left of both graphs illustrates the critical importance of selecting the proper interval for sample collection as well as the problem of measuring the data on a discrete scale rather than a continuous scale. Bleeding the calves at weekly intervals rather than biweekly or monthly intervals for

TABLE 3.1

Comparison of preparturient and parturient AGID test results of 27 dams to the 1st negative AGID test results of their 27 calves.

Day of Sample*	No. of Cows	Test Result (cow)	Day of 1st (-) AGID TEST (calf)
MINUS 42	27(100.00)	+	NA**
Plus 1	13(48.14)	+	Plus 147
Plus 1	5(18.15)	W+	Plus 70
Plus 1	9(33.71)	-	Plus 56

*Relative to calving data. minus = preparturient; plus = postparturient.

**NA = not applicable. ( ) = % of total tested.

the first 4 weeks would provide a partial solution to the problem of having a sampling interval of 14 days. Shortening the sampling interval also would delete the problem of excluding calves with less than 3 positive observations. The size of the study group would increase considerably and the data would allow for a more accurate statistical analysis.

Statistical theory indicates that the validity of these pooled estimates has a direct association with the number of calves sampled and with the number of positive observations per calf (Snedecor and Cochran, 1967). However, this theory contradicted the biological behavior of the calves used in this study since less than 3 positive observations were obtained from several calves. Because only a small percentage of the clients who were solicited agreed to participate, these results should not be viewed as representative of a large number of calves.

The manner in which these 4 herds was selected was via convenience sampling. Clinical lymphosarcoma had been documented in each of the herds. Ideally, a random sampling procedure should have been used. Because nonrandom sampling was done, the calves are not representative of the entire population of Michigan dairy calves. However, a BLV vaccination program presently is of major concern to herds in which clinical disease is severe and the

antibody prevalence is high, or in which exportation is of importance. Since herds uncharacteristic of these 2 groups probably will not have BLV antibodies, they simply cannot be included in a study of this type. The reference population can include only those herds with BLV antibodies. Thus, the study has external validity for that population.

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CONCLUSIONS

These 3 studies dwelled on the terminal phases of study of BLV in relation to its effects on the cattle industry. A huge body of knowledge concerning the etiology, transmission, pathology, physiological alterations, affects on the immune system and more about BLV has accumulated since the discovery of the virus. The present areas of major concern, areas which are crucially important economically to the cattle industry, are control, prevention and eradication of BLV. The knowledge gained from these studies hopefully will help to fill existing voids in these 3 areas.

General recommendations for the control of BLV can be based on these and other studies. One must bear in mind that the practical applicability of these recommendations will vary with a given setting. Additionally, though most of the recommendations are based on scientific studies, it was necessary to base a few recommendations on logical assumptions or empirical conclusions. The validity of any of the recommendations may change if future research produces results which differ from the results of these studies.

RECOMMENDATIONS FOR CONTROL OF BOVINE LEUKEMIA VIRUS

1. All animals at least 6 months of age should be serotested using the AGID test. The age of initial serotesting may be reduced to as early as 3 months if future research shows that BLV colostral antibodies decay before 6 months of age.

2. A 3-month to 4-month herd serotesting interval may be more appropriate since seroconversions occurred so infrequently using the monthly testing schedule in this study.

3. Serotesting of pregnant animals should be done at least 6 weeks prior to parturition to prevent false-negative results due to immunoglobulin shifts.

4. If facilities allow, physically separate the BLV positive animals from the negative animals.

5. Identify positive and negative animals with different colored ear tags.

6. Breeding establishments should require isolation of purchased cattle followed by 2 consecutively negative serotests at least 4 weeks apart.

7. Animals to be exported to countries requiring AGID negative tests ideally should be exported prior to 16 months of age or kept separated from positive cows until they are exported.

8. Seronegative calves should be isolated from seropositive cows.

9. Male calves not being retained as sires or as steers should be sold at an early age.

10. If they are retained and require castration, an appropriate disinfectant should be used on the surgical instruments after each castration.

11. Calves can be fed colostrum from BLV positive cows to obtain BLV antibodies. However, they should be fed milk from BLV negative cows to prevent infections via milk cells.

12. Milk from the bulk tank may be fed safely if BLV viral particles are diluted sufficiently by milk from negative cows.

13. Older, low-producing seropositive cows in commercial dairies should be culled.

14. Seronegative cows should be milked first.

15. The milking procedures should prevent the contact of positive and negative cows.

16. Cows with udder disease leading to the deposition of blood on the milking equipment should be milked at least temporarily using different milking equipment.

17. Disinfect dehorning equipment after dehorning each animal. Electrocautery dehorners are useful for cauterizing bloody dehorning sites, irrespective of the method used to remove the horn buds.

18. A good quality insect control program should be instituted.

19. All personnel should be educated and reminded constantly of the importance of following these guidelines.

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