MASTER

RECEIVED BY DTIC MAY 25 1970

EPIDEMIOLOGICAL STUDIES ON THE NATURAL TRANSMISSION OF BOVINE LEUKEMIA

V. L. Larson, D.V.M., Ph.D., D. K. Sorensen, D.V.M., Ph.D., R. K. Anderson, D.V.M., M.P.H., and V. Perman, D.V.M., Ph.D.

From the Department of Veterinary Medicine (Larson and Sorensen), Department of Veterinary Microbiology and Public Health (Anderson), and Department of Veterinary Pathology and Parasitology (Perman), College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota 55101.

This investigation was supported in part by the United States Atomic Energy Commission under Contract No. AT(11-1) - 910.

Published as paper number _____, Scientific Journal Series, Minnesota Agricultural Experiment Station, University of Minnesota, St. Paul, Minnesota.

LEGAL NOTICE

This report was prepared as an account of Government sponsored work. Neither the United States, nor the Commission, nor any person acting on behalf of the Commission:

A. Makes any warranty or representation, expressed or implied, with respect to the accuracy, completeness, or usefulness of the information contained in this report, or that the use of any information, apparatus, method, or process disclosed in this report may not infringe privately owned rights; or

B. Assumes any liabilities with respect to the use of, or for damages resulting from the use of any information, apparatus, method, or process disclosed in this report.

As used in the above, "person acting on behalf of the Commission" includes any employee or contractor of the Commission, or employee of such contractor, to the extent that such employee or contractor of the Commission, or employee of such contractor prepares, disseminates, or provides access to, any information pursuant to his employment or contract with the Commission, or his employment with such contractor.

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

The cause of bovine leukemia* is unknown. Most of the present knowledge of this disease has been gained through epidemiological studies. These studies have shown that the disease occurs with high incidence in certain geographic areas (1,3,8,27,31), enzootically in herds (2,9,16,24), and in particular families within herds. (4,11,13,30). Several investigators have also reported observing persistent lymphocytosis in normal cattle in high incidence leukemia herds. (1,2,7,8,10,14,15,16,19,22,26,28,31) A strong association between pre-clinical persistent lymphocytosis (P.L.) and the development of clinical leukemia has been reported from studies of leukemia herds. (2,7,16,28,32)

The appearance of bovine leukemia in certain geographic areas and herds may represent foci of infections if, as is believed by most investigators, the disease is caused by a virus. Similarities between bovine leukemia and the viral avian, feline, and murine leukemias also support a viral etiology. While the reported significant familial aggregations of leukemia cases have been interpreted by some as evidence of genetic predisposition, vertical viral transmission could account for these aggregations.

* Bovine leukemia refers to neoplastic proliferation of lymphoreticular tissue and is synonymous with bovine leukosis and lymphosarcoma as used by other investigators.

Reports of etiological studies on bovine leukemia i.e., viral isolations and transmission experiments, have been encouraging but as yet inconclusive. (6,12,18,21,23,25,29) Definitive proof of etiology must await clearly defined and well controlled successful transmission experiments using specific isolates.

The present report deals primarily with clusters of leukemia cases which had common neonatal associations (neonatal cohorts). These close temporal-spatial relationships were examined for evidence of natural transmission of bovine leukemia at an early age, which would be consistent with the demonstrated increased susceptibility of young chickens and mice to leukemia viruses.

Materials and Methods

Eleven herds were studied in which two or more adult cases of bovine leukemia had been diagnosed and which had reliable pedigree and birth date information. Eight herds were Holstein, one was Jersey, one Red Polled, and one Milking Shorthorn. The number of adult cattle in the herds ranged from 16 to 100 with an average of 48 cows. The leukemia cases were all of adult ages and the diagnoses were confirmed at necropsy.

Hematological studies were conducted semiannually on all cattle 2 years of age and older in the herds for periods ranging from 4 to

7 years. Total leukocyte counts were done on blood preserved with sodium ethylenediaminetetra-acetic acid in a electronic particle counter.* Two hundred cell differential counts were made of freshly prepared Wright's stained blood films. A minimum of one year of study and two hematological examinations were required for data analyzed.

The animals are dichotomized into persistent lymphocytotic and nonlymphocytotic groups by use of Bendixen's "Lymphocyte Key." Hematologic parameters recently established for Minnesota cattle are essentially the same as those reported by Bendixen. Twenty one cases of leukemia are analyzed with the P.L. group (15 dams and 6 daughters) because they exhibited P.L. prior to the development of clinical leukemia (i.e. appearance of tumors).

The term neonatal cohort as used in this paper connotes an adult group of cattle which were born in a common 28 day period of time. For presentation purposes the neonatal cohorts are designated by the pre-dominant month of birth for each cohort.

Results

Cohort Studies -- Birth records of 11 high incidence leukemia herds were studied. In 7 herds 2 or more adult leukemia cases, in each herd, had been born in a common 4 week period (Table 1). Four of these herds (2, 27, 31, and 39) had 2 leukemia cases each,

2 herds (1 and 32) had 3 leukemia cases each and one herd (23) had 4 leukemia cases born within a 4 week period. In 5 herds the birth date intervals for the leukemia cases were 2 weeks or less.

The 7 herds with high leukemia incidence neonatal cohorts had a total of 30 cases of leukemia diagnosed. That 18 (60%) of the cases were in high leukemia incidence neonatal cohorts indicates how common this type of clustering was in these herds.

It is of interest that although frequently, 2 or more leukemia cases had close proximity of birth, their dates of death due to leukemia were, with two exceptions, quite variable. In 5 herds, the leukemia cohort members had differences of dates of death ranging from 10 to 45 months. The 2 cases in Herd 2 developed clinical leukemia simultaneously, at 6 years of age. The 3 cases in Herd 1 all exhibited initial signs of tumors within a one month period at 4 years of age.

Additional examination of records on all adult cattle studied in 11 herds revealed neonatal cohort clustering of animals which exhibited persistent lymphocytosis. High incidences of P.L. in adult cattle ^{even} was especially prominent in those neonatal cohorts where one or more adult members had died of leukemia. (Table 1). There were 64 adult cows available for study which were born in 14 neonatal cohorts. Twenty five (39%) of these developed leukemia and an additional 31 (48%) exhibited P.L. during adult life.

The appearance of the neonatal cohort clusters of leukemia cases and animals with P.L. was very spectacular since other neonatal cohorts born in the same years on these farms had few animals which exhibited lymphocytosis. Investigations to determine the differential exposure experiences of high and low incidence cohorts revealed that the high cohorts invariably had contained progeny of dams which had died of leukemia or exhibited P.L. Furthermore, several of these dams were in the clinical tumor stage of leukemia when these progeny were born.

Management practices on the 11 farms were such that, during the neonatal period, calves in each neonatal cohort had direct contact with each other and common sources of food. This was the case due to practices of limited age segregation in pens and pooling of milk or fresh colostrum. Therefore, each calf during the neonatal period had exclusive contact with all of their particular neonatal cohort dams (including the leukemia cases) through close association with their progeny, colostrum, and milk.

Familial Studies -- The role of possible vertical transmission of an agent or genetic susceptibility in the causation of leukemia and P.L. was studied through comparison of data from long term hematologic study of 310 dam-daughter pairs in high incidence leukemia herds. These data show a significant ($P=.001$) similarity between dams and their daughters on the basis of leukemia and lymphocyte

TABLE 2

Analysis of the Difference Between Presence of
Lymphocytosis and Leukemia in
Dam-Daughter Pairs

	Dams		
	Non- lymphocytotic	Lymphocytotic and/or Leukemia	Total
<u>Daughters</u>			
Nonlymphocytotic			
Observed	164	41	205
Expected	152	53	
Lymphocytotic or Leukemic			
Observed	66	39	105
Expected	<u>78</u>	<u>27</u>	<u> </u>
TOTAL	230	80	310

$$\chi^2 = 10.84 \text{ 1 d.f.}$$

$$p = .001$$

counts (Table 2). Of the dams which developed leukemia or those which exhibited P.L. 39/80 (49%) had daughters which exhibited P.L. or died of leukemia. The cows with lymphocyte counts in the normal range had only 66/230 (29%) of their daughters which exhibited P.L. or died of leukemia.

The 66 (29%) P.L. daughters of cows with normal lymphocyte counts could not be satisfactorily attributed to sire effect. Examination of birth dates on these daughters did reveal that 53/66 (80%) of them to be members of high incidence leukemia or P.L. neonatal cohorts. Therefore it would seem more likely to implicate the common neonatal environment as the major factor in causation rather than genetic effect.

Discussion

The exact significance of neonatal cohort clustering in the seven leukemia clusters observed in this sample of cases is not yet clear. Therefore, conclusions based on the inferred implications of these occurrences must remain speculative in a study of this type. But since the neonatal cluster represents a random genetic sample drawn from the herd, i.e. that the members of the cohort were not unusually closely related genetically nor unusually genetically different from other cattle in the herds, then some form of transmission is apparent.

The existence of such close temporal-spatial relationships between two or more leukemia cases in seven different herds indicates that transmission occurs under very special circumstances. The neonatal clustering strongly implies that there is age specific or age limited susceptibility in the natural transmission of bovine leukemia. The close temporal-spatial relationships of these cases also implicates a differentially specific source of exposure for these cohorts during the neonatal period.

It is interesting to speculate on all of the numerous possible exposure experiences that the leukemia cases had as calves. Those potential environmental exposures which are common to all cattle in the herds must naturally be rejected. Subsequently, only those common exposure experiences which can be implicated are those which were specific for certain groups of cattle during the neonatal period and not for other cattle in the herds born during the same year.

The presence of progeny of leukemia case and P.L. dams in the high incidence cohorts indicates that there is a potential direct source of the "bovine leukemia virus" via calf or infective lactate. The herd management practices of pooling milk or colostrum does give certain neonatal cohorts indirect contact with these potential carriers of infection during the milk consuming period of life. These possible modes of both vertical and horizontal transmission may be analogous

to that seen in murine leukemia where virus has been demonstrated in milk (5) and transmission via milk has been accomplished in foster nursing experiments. (17,20)

It is interesting that the leukemia cases in two cohorts were clustered temporally both with respect to birth and also at death. The lack of temporal clustering at death in the other five cohorts may indicate individual variation in the induction period of leukemia. This is possibly due to variation in susceptibility or other unknown promoting factors.

Neonatal association with calves which developed leukemia as adults evidently appears to be associated with occurrence of P.L. If the P.L. seen in cattle of leukemia herds is a preclinical stage of leukemia then these associations have added significance. This study group has reported a strong association between P.L. and the development of leukemia in Minnesota cattle. Similar findings have been reported elsewhere.

In view of the evidence indicated by these data a hypothesis that involves both vertical and horizontal transmission of bovine leukemia may be postulated. Familiar similarities of lymphocyte counts in dam-daughter pairs seen in this study and by others suggests a possibility of vertical transmission or genetic susceptibility in the causation of P.L. Additionally, the reported occurrences of

familial aggregations of leukemia cases also suggests vertical transmission of an agent or genetic susceptibility or both as possible mechanisms in leukemia causation. Since all studies of this type are retrospective epidemiologic investigations it is impossible to attribute these occurrences to either in utero infection or genetic effect.

The paucity of significant familial aggregations, when viewing the large leukemia case populations studied, suggests a significant role for horizontal transmission in addition to vertical modes in leukemia transmission. The dissimilarities seen in this study between normal dams and some of their daughters in respect to lymphocyte counts and leukemia may be largely explained by the hypothesis of horizontal transmission during the neonatal period.

Summary

Examination of animal records in high incidence bovine leukemia herds revealed a close neonatal relationship between adult leukemia cases. In 7 herds 2 or more cases of leukemia were born within 4 weeks of each other. High incidences of persistent lymphocytosis were also observed in adult cattle born in common neonatal periods with adult leukemia cases in 11 herds.

Studies of dam-daughter pairs in respect to leukemia and lymphocytosis incidence revealed significant familial similarities. Daughters which were dissimilar to their dams had neonatal association with leukemia cases.

These studies indicate that bovine leukemia may be naturally transmitted vertically to progeny and also horizontally to susceptible neonatal calves.

References

¹Bendixen, H. J.: Undersøgelser over Kvaegets Leukose. I. Kvaegleukosens Forekomst og Uabredelse i Danmark. Nord. Vet. Med., 9, (1957): 1-33.

²Bendixen, H. J.: Studies of Leukosis Enzootica Bovis. U.S. Dept. of Health, Ed. and Welfare; Public Health Service Publication No. 1422, Washington, D. C., 1965.

³Conner, G. H., La Belle, J. A., Langham, R. F., and Crittenden, M.: Epidemiology of Bovine Leukemia Jour. of Nat. Cancer Inst., 36, (1966): 383-388.

⁴Croshaw, J. E., Abt, D. A., Marshak, R. R., Hare, W. C. D., Switzer, J. W., Ipsen, J., and Dutcher, R. M.: Pedigree studies in bovine lymphosarcoma. Ann. N.Y. Acad. Sci., 108, (1963): 1193-1202.

⁵Dmochowski, L.; Grey, C. E.; Padgett, F. and Sykes, J. A.: Studies on the structure of the mammary tumor-inducing virus and leukemia virus. Viruses, Nucleic Acids and Cancer. Proc. Univ. of Texas, 85-121 (Univ. of Texas Press, Houston 1963).

⁶Dutcher, R. M.: Viral Research on Bovine Leukosis. Proc. 3rd int. Symp. comp. Leukemia Res., Paris 1967; Bibl. haemat. No. 31, 116-135 (Karger, Basel/New York 1968)

⁷Egehoj, J.: Some Experimental Investigations on the Etiology of Leucemia in Cattle. Amer. J. Vet. Res., 8, (1947): 57-65.

⁸Götze, R., Rosenberger, G., and Ziegenhagen, G.: Die Leukose des Rindes. Ihre hämatologische und klinische Diagnose. Mh. Vet. Med., 9, (1954): 517-526.

⁹Götze, R., Rosenberger, G., and Ziegenhagen, G.: Über Ursachen und Bekämpfung der Rinderleukose. II Weitere Bemerkungen zur Diagnose und zur Erbllichkeit. Dtsch Tierärztl. Wschr., 62, (1955): 353-357.

- ¹⁰Hare, W. C. D., Marshak, R. R., Abt, D. A., Dutcher, R. M., and Croshaw, J. E.: Bovine Lymphosarcoma. A Review of Studies on Cattle in the Eastern United States. *Can. Vet. J.*, 5, (1964): 180-198.
- ¹¹Henricson, R. and Olson, H.: Statistische Untersuchungen Über Die Rinder Leukose, *Acta Vet Scand.*, 2, Sup. 2, (1961): 55-62.
- ¹²Hoflund, S., Thorell, B. and Winqvist, G.: Experimental transmission of bovine leukosis. *Proc. Int. Sym. an Comp. Leuk. Res.*, Hanover, (1963).
- ¹³Karlson, A. G.: Clinical and Postmortem Observations on Lymphoblastoma of Cattle. Ph. D. Thesis, Univ. of Minnesota., 1942.
- ¹⁴Knuth, P., and Volkmann, O.: Untersuchungen Über die Lymphozytomatoses des Rindes. *Ztschr. Infkr. Haustiere.*, 17, (1916): 393-467.
- ¹⁵Knuth, P. and du Toit, P. J.: Weitere Untersuchungen über die Lymphozytomatose des Rindes. *Berl. Tierärztl. Wschr.*, 33 (1917): 205-208.
- ¹⁶Larson, V. L.: Bovine Leukemia. A Clinical, Epidemiological, and Experimental Study. Ph. D. Thesis, University of Minnesota, 1968.
- ¹⁷Law, L. W.: Influence of foster-nursing on virus-induced and spontaneous leukemia in mice. *Proc. Soc. exp. Biol. Med.* 3: 615-623 (1962).
- ¹⁸Malmquist, W. A., Van Der Maaten, M. J., and Boothe, A. D.: Isolation, Immuno-diffusion, Immunofluoresence, and Electron Microscopy of a Syneytial Virus of Lymphosarcomatous and Apparently Normal Cattle. *Cancer Research* 29, (1969): 188-200.
- ¹⁹Marshak, R. R., Hare, W. C. D., Abt, D. A., Croshaw, J. E., Switzer, J. W., Ipsen, I., Cutcher, R. M., and Martin, J. E.: Occurrence of Lymphocytosis in Dairy Cattle Herds with High Incidence Lymphosarcoma. *Ann. N. Y. Acad. Sci.* 108, (1963): 1289-1301.
- ²⁰Mirand, E. A.; Buffett, R. F. and Grace, J. T.: Mode of transmission of Friend virus disease. *Proc. Soc. exp Biol. Med.* 121: 970-976 (1966).

²¹Montemagno, F.: Trasmissibilita in serie della lencogi linfatica sperimentale dei bovini, Acta Med. Vet., 9, (1963): 151-158.

²²Niepage, H.: Zur Diagnose der Rinderleukose durch den Leukose-schlüssel. Berl. Munch Tierärztl. Wschr., 67, (1954): 253-255.

²³Papparella, V., Cali, A., Rossi, G. B., and Iacobelli, A.: Researches on a Virus Isolated from a Calf Affected with Lymphatic Leukemia. Ann. New York Acad. Sci., 108, (1963): 1173-1192.

²⁴Rosenberger, G.: Studies on Bovine Leukosis in Germany. II. Occurrence and Epidemiology, WHO Conf. on Comparative Studies in Leukemias. Philadelphia, Pa., (1961).

²⁵Rosenberger, G.: Successful transmission trials of leucosis in Cattle. Proc. Int. Symp. on Comp. Leuk. Res. Hannover. (1963).

²⁶Seelemann, M., Heeschen, W., Kreuger, K. E., and Brunies, A.: Untersuchungen über die Leukose des Rindes. Kiel. Mitchwirtsch. Forsch Ber. 16, (1964): 1-17.

²⁷Sorensen, D. K., Anderson, R. K., Perman, V., and Sautter, J. H.: Studies of Bovine Leukemia in Minnesota. Nord. Vet-Med. Suppl. I. (1964): 562-572.

²⁸Theilen, G. H., Dungworth, D. L., Lengyel, J., and Rosenblatt, L. S.: Bovine Lymphosarcoma in California. 1. Epizootiologic and Hematologic Aspects. Health Lab. Sci. 1, (1964): 96-106.

²⁹Theilen, G. H., Dungworth, D. L., Harrold, J. B., and Straub, O. C.: Bovine Lymphosarcoma Transmission Studies. Am. J. Vet. Res., 28, (1967): 373-386.

³⁰Verter, W., and Gehrke, E.: Die Ausbreitung der Leukose in Kuhfamilien Mh. Vet. Med., 20 (1965): 533-537.

³¹Wiesner, E.: Die Leukose des Rindes. Jena, (1961).

³²Winqvist, G.: Some haematological observations on bovine leukosis in Sweeden during the years 1956-1963. Prac. Int. Symp. on Comp. Leuk. Res. Hannover. (1963).