Europ. J. Cancer, Vol. 15, pp. 1395–1400

0014-2964/79/1101-1395 $02.00/0

Influence of Genetic Factors on the Susceptibility of Cattle to Bovine Leukemia Virus Infection*

M. J. BURRIDGE,†‡ C. J. WILCOX§ and J. M. HENNEMANN†

†*Department of Preventive Medicine, College of Veterinary Medicine and* §*Dairy Science Department, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida* 32610, *U.S.A.*

Abstract—*An epidemiological study of a dairy herd in Florida was undertaken to investigate the factors that influence infection with bovine leukemia virus (BLV). Using the glycoprotein antigen immunodiffusion test,* 75.8–82.7% *of the cows were found to have antibodies to BLV between* 1975 *and* 1977. *Prevalence of BLV antibodies increased until* 5 yr *of age after which it remained at a steady level. The data were examined by least squares analysis of variance to determine heritability of susceptibility to BLV infection. A heritability estimate of* 0.48 ± 0.22 *was determined, suggesting considerable genetic influence on susceptibility to BLV infection.*

INTRODUCTION

BOVINE leukemia virus (BLV) has many morphological, biochemical and biophysical similarities with the other mammalian and avian leukemia viruses [1–10]. BLV particles have been identified repeatedly in cultures of lymphocytes from cattle with lymphosarcoma [1, 3–5, 11–13]. Transmission experiments have shown that BLV has oncogenic activity [14–16]. Sero-epidemiological studies have demonstrated a strong association between BLV infection and bovine lymphosarcoma [17–20] and have indicated that the predominant mode of transmission of BLV is horizontal [21, 22]. Electron microscopical studies detected BLV in 19 out of 20 fluorescent antibody-positive cattle but failed to demonstrate the virus in 12 antibody-negative animals [19]. As a result of these findings, it is widely accepted that BLV is causally related to bovine lymphosarcoma, a systemic malignancy also referred to as bovine leukemia and bovine leukosis [23].

Accepted 15 May 1979.

*Supported by Biomedical Research Support Grant no. RR05788-01 and by a grant from Pitman–Moore, Inc., Washington Crossing, New Jersey, U.S.A.

‡Reprint requests to: Dr. M. J. Burridge, College of Veterinary Medicine, Box J-136, University of Florida, Gainesville, Florida 32610, U.S.A.

Many epidemiological investigations have examined the influence of various factors on infection of cattle with bovine lymphosarcoma or BLV. Studies on clinical cases have suggested that the prevalence of bovine lymphosarcoma is related to age [24–29], breed [25, 29], type of production (dairy or beef) [25, 27], and herd size [26, 28, 29]. Serological studies have found that the prevalence of antibodies to BLV antigens is similarly related to age [18, 22, 30] and type of production [30, 31], but not herd size [30, 31]. In addition, familial aggregations of cases of bovine lymphosarcoma have been demonstrated [11, 32–38], suggesting the possibility that genetic factors might influence the susceptibility of cattle to the disease.

The present study was undertaken to investigate the factors that influence infection of dairy cattle with BLV and, in particular, to measure the extent to which susceptibility to BLV infection is inherited in the cow.

MATERIALS AND METHODS

Cattle

The cattle studied were those cows in the University of Florida Dairy Research Unit herd which were 18 months of age or older. The composition of the herd by breed was 56% Holstein, 31% Jersey, 7% Holstein

crosses, 4% Guernsey and 2% Brown Swiss. The animals ranged in age from 18 months to 13 yr old. The cows were bled for serum collection as follows: 227 animals in October 1975, 225 in August 1976 and 219 in August 1977. Six cases of bovine lymphosarcoma had been confirmed histopathologically in the herd between September 1976 and June 1978. The breed and age of each animal were recorded at the time of bleeding. Furthermore, the sire of each cow was identified, all cattle in the herd having been sired by artificial insemination.

Serology

Each serum sample was tested for BLV antibodies using the agar gel immunodiffusion test with a glycoprotein antigen (Leukassay-B, Pitman–Moore Inc., Washington Crossing, New Jersey). Agar gel plates were prepared with 0.7% Noble agar (Difco Laboratories, Detroit, Michigan) in 0.15M borate buffer, pH 8.6, containing 7% sodium chloride. Fifteen millilitres of agar were dispensed into each 100 mm Petri dish. One central and 6 peripheral wells, each 7 mm in diameter and 3 mm apart, were cut in each quadrant of the Petri dish, allowing for 1 control pattern and 3 test patterns per dish. Each pattern of wells consisted of a central antigen well surrounded by 6 equidistant serum wells. For the control pattern, the serum wells were filled with negative, weak positive or strong positive control sera. For each test pattern, they were filled alternately with positive control serum and test sera. The plates were incubated for 48 hr in a humidified chamber at room temperature. Test sera were recorded as positive when precipitation lines of identity formed with positive control serum. Each positive reaction was interpreted to indicate past infection with BLV. This test was chosen for the present study because of its simplicity and because of its high relative sensitivity and relative specificity when compared with established tests for the serodiagnosis of BLV infection [39–41].

Data analysis

The dairy herd data were examined by least squares analysis of variance to determine the heritability of susceptibility to BLV infection in the cow. Statistical analysis was performed by use of Harvey's Least Squares Maximum Likelihood Mixed Model computer program [42], with the model:

$$y_{ijklm} = \mu + a_i + b_{ij} + c_{ijk} + F_l + e_{ijklm}$$

where y_{ijklm} represents the test result for BLV antibodies, μ the mean, a_i the breed of the cow (fixed effect), b_{ij} the sire of the cow (random nested effect), c_{ijk} the cow herself (random nested effect), and F_l other fixed effects (linear, quadratic and cubic terms for age). The model, therefore, was a nested design (daughters nested within sires and sires nested within breeds) with cross-classified fixed effects. Heritability, as 4 times the paternal half-sib correlation, was estimated using the formula:

$$\text{Heritability} = \frac{4\sigma_s^2}{\sigma_c^2 + \sigma_s^2 + \sigma_e^2}$$

where σ_s^2 was the sire variance, σ_c^2 the variance among daughters within sires, and σ_e^2 the error variance representing the variability among observations within daughters within sires. Heritability is defined as the ratio of additive genetic variance to phenotypic variance [43]. It measures the fraction of the total phenotypic variance due to variation in breeding values of individuals, the breeding value of any individual being equal to the sum of the average effects of the genes it carries [44].

RESULTS

The results of the least squares analysis of variance are summarized in Table 1. Prevalence of BLV antibodies was significantly influenced by sire (nested within breed), cow (nested within sire within breed) and age, but not by breed. Variance component estimates were: $\sigma_s^2 = 0.01691$, $\sigma_c^2 = 0.06627$, and $\sigma_e^2 = 0.05791$. Heritability thus was 0.48 (± 0.22 S.E.).

The prevalence of BLV antibodies in the dairy herd was 78.9% (179 of 227 seropositive) in 1975, 82.7% (186 of 225) in 1976 and 75.8% (166 of 219) in 1977. The regression of prevalence of BLV antibodies on age is shown in Fig. 1. The prevalence increased from 18 months to 5 yr of age and thereafter remained at a consistently high level of about 90%.

DISCUSSION

It was demonstrated over 30 yr ago that genetic factors influence the incidence of leukemia in mice [45, 46]. Later, it was found that susceptibility of mice to leukemogenesis by Gross virus was genetically determined [47, 48]. Many investigators have reported familial aggregations of cases of leukemia in man [49–56] and cattle [11, 32–38]. It was concluded that the aggregations of human cases probably resulted from a genetic predisposition to leukemia [55, 56]. The results of the bovine

Table 1. Least squares analysis of variance showing factors influencing prevalence of BLV antibodies in Florida dairy cows, 1975–1977

Source of variation	Degrees of freedom	Mean squares	*F*-ratio
Breed	4	0.379	1.20
Sire within breed	79	0.316	1.73*
Cow within sire within breed	265	0.183	3.15*
Age (linear)	1	1.174	20.28*
Age (quadratic)	1	0.589	10.17*
Age (cubic)	1	0.344	5.94†
Error	319	0.058	

*$P<0.01$.
†$P<0.025$.

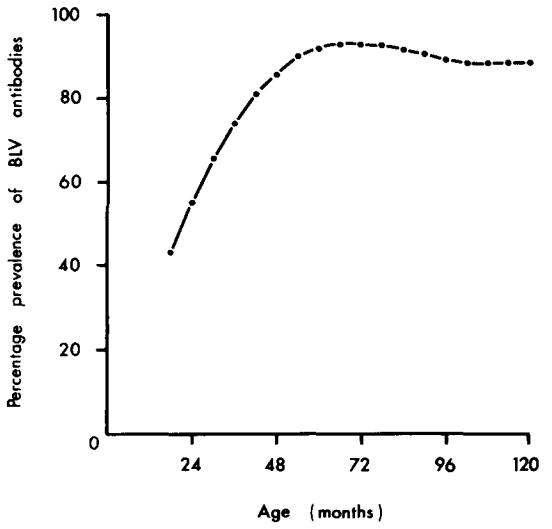

Fig. 1. Regression of prevalence of BLV antibodies on age in a Florida dairy herd, 1975–1977.

studies were interpreted to suggest either genetic susceptibility to bovine lymphosarcoma or vertical transmission of an infectious agent [33–36]. Subsequent work indicated that BLV, the putative infectious agent of bovine lymphosarcoma, was transmitted horizontally in most instances [21, 22]. There are ample data, therefore, to warrant further investigation of genetic factors as host determinants of human and bovine leukemia.

This study used least squares analysis of variance procedures to examine for any influence of genetic factors on infection with BLV in a dairy herd. Heritability was estimated from the paternal half-sib correlation, a method that minimized non-genetic biases by consideration of only sire effects. As a result, the only possible non-genetic influence on the heritability estimate would have been transmission of infection via frozen semen from sire to dam to fetus. However, recent studies have indicated no evidence of transmission of BLV from infected bulls to offspring sired by artificial insemination [57, 58]. The results, therefore, provide convincing evidence of genetic susceptibility to BLV infection and, consequently, to bovine lymphosarcoma. The heritability of 0.48 suggests that this trait is quite highly heritable. For comparison, the milk yield of cattle has a heritability value of about 0.25 [59].

The relatively large S.E. of 0.22 arises from the small sample size (671 observations on 349 cows by 84 sires) and the analysis of binary data (positive and negative test reactions being coded 1 and 0, respectively). Assuming the heritability estimate to be unbiased, the standard error suggests that there is about a two-third's chance that the true value of heritability falls between 0.26 and 0.70 (0.48 ± 0.22).

Only a small proportion of the cattle that develop detectable antibodies to BLV manifest clinical disease [20], demonstrating that BLV is only one of the determinants of bovine lymphosarcoma. The heritability study reported herein suggests that genetic factors may be another of the multiple determinants of the disease since they have been shown to influence infection of cattle with BLV. This study also examined other variables (breed and age) for their influence on BLV infection.

There was no significant difference ($P>0.05$) in prevalence of BLV antibodies between the different breeds in the dairy herd. However, there was a well-defined relationship between prevalence of BLV antibodies and age. Prevalence progressively increased until 5 yr of age after which it remained at a steady level. Similar age trends have been demonstrated in other serological studies [18, 22, 30]. Although no cattle less than 18 months old were examined in this study, the

data did indicate that many animals became infected after calfhood, supporting horizontal transmission as the major mode of spread of BLV.

The heritability study reported in this paper considered only 1 dairy herd and, therefore, needs to be repeated by examination of an appropriate sample of herds from a wider geographical area. Nevertheless, the initial results indicate that the susceptibility of cattle to BLV infection is partially under genetic control, suggesting genetic selection as a possible method for control of bovine lymphosarcoma.

Acknowledgements—The authors are indebted to Dr. H. N. Becker of the College of Veterinary Medicine, University of Florida, for provision of the serum samples.

REFERENCES

1. J. M. Miller, L. D. Miller, C. Olson and K. G. Gillette, Virus-like particles in phytohemagglutinin-stimulated lymphocyte cultures with reference to bovine lymphosarcoma. *J. nat. Cancer Inst.* **43,** 1297 (1969).
2. T. G. Kawakami, A. L. Moore, G. H. Theilen and R. J. Munn, Comparisons of virus-like particles from leukotic cattle to feline leukosis virus. *Bibl. Haemat. (Basel)* **36,** 471 (1970).
3. J. F. Ferrer, N. D. Stock and P. Lin, Detection of replicating C-type viruses in continuous cell cultures established from cows with leukemia: effect of the culture medium. *J. nat. Cancer Inst.* **47,** 613 (1971).
4. L. D. Miller, J. M. Miller and C. Olson, Inoculation of calves with particles resembling C-type virus from cultures of bovine lymphosarcoma. *J. nat. Cancer Inst.* **48,** 423 (1972).
5. N. D. Stock and J. F. Ferrer, Replicating C-type virus in phytohemagglutinin-treated buffy-coat cultures of bovine origin. *J. nat. Cancer Inst.* **48,** 985 (1972).
6. J. Calafat, P. C. Hageman and A. A. Ressang, Structure of C-type virus particles in lymphocyte cultures of bovine origin. *J. nat. Cancer Inst.* **52,** 1251 (1974).
7. R. V. Gilden, C. W. Long, M. Hanson, R. Toni, H. P. Charman, S. Oroszlan, J. M. Miller and M. J. Van der Maaten, Characteristics of the major internal protein and RNA-dependent DNA polymerase of bovine leukemia virus. *J. gen. Virol.* **29,** 305 (1975).
8. R. Callahan, M. M. Lieber, G. J. Todaro, D. C. Graves and J. F. Ferrer, Bovine leukemia virus genes in the DNA of leukemic cattle. *Science* **192,** 1005 (1976).
9. R. Kettmann, D. Portetelle, M. Mammerickx, Y. Cleuter, D. Dekegel, M. Galoux, J. Ghysdael, A. Burny and H. Chantrenne, Bovine leukemia virus: an exogenous RNA oncogenic virus. *Proc. nat. Acad. Sci. (Wash.)* **73,** 1014 (1976).
10. F. Weiland and S. Ueberschar, Ultrastructural comparison of bovine leukemia virus (BLV) with C-type particles of other species: brief report. *Arch. Virol.* **52,** 187 (1976).
11. C. Olson, L. D. Miller, J. M. Miller and K. G. Gillette, Progress on transmission of bovine lymphosarcoma. *Bibl. Haemat. (Basel)* **36,** 476 (1970).
12. S. K. Dutta, V. L. Larson, D. K. Sorensen, V. Perman, A. F. Weber, R. F. Hammer and R. E. Shope, Isolation of C-type virus particles from leukemia and lymphocytotic cattle. *Bibl. Haemat. (Basel)* **36,** 548 (1970).
13. M. J. van der Maaten, J. M. Miller and A. D. Boothe, Replicating type-C virus particles in monolayer cell cultures of tissues from cattle with lymphosarcoma. *J. nat. Cancer Inst.* **52,** 491 (1974).
14. C. Olson, L. D. Miller, J. M. Miller and H. E. Hoss, Transmission of lymphosarcoma from cattle to sheep. *J. nat. Cancer Inst.* **49,** 1463 (1972).
15. C. Olson and L. E. Baumgartener, Pathology of lymphosarcoma in sheep induced with bovine leukemia virus. *Cancer Res.* **36,** 2365 (1976).
16. M. J. van der Maaten and J. M. Miller, Induction of lymphoid tumors in sheep with cell-free preparations of bovine leukemia virus. *Bibl. Haematol.* **43,** 377 (1976).
17. J. F. Ferrer, L. Avila and N. D. Stock, Serological detection of type C viruses found in bovine cultures. *Cancer Res.* **32,** 1864 (1972).

18. C. Olson, H. E. Hoss, J. M. Miller and L. E. Baumgartener, Evidence of bovine C-type (leukemia) virus in dairy cattle. *J. Amer. vet. med. Ass.* **163,** 355 (1973).
19. J. F. Ferrer, D. A. Abt, D. M. Bhatt and R. R. Marshak, Studies on the relationship between infection with bovine C-type virus, leukemia, and persistent lymphocytosis in cattle. *Cancer Res.* **34,** 893 (1974).
20. J. F. Ferrer, D. M. Bhatt, R. R. Marshak and D. A. Abt, Further studies on the antigenic properties and distribution of the putative bovine leukemia virus. *Bibl. Haemat. (Basel)* **40,** 59 (1975).
21. C. E. Piper, D. A. Abt, J. F. Ferrer and R. R. Marshak, Sero-epidemiological evidence for horizontal transmission of bovine C-type virus. *Cancer Res.* **35,** 2714 (1975).
22. J. F. Ferrer, C. E. Piper, D. A. Abt, R. R. Marshak and D. M. Bhatt, Natural mode of transmission of the bovine C-type leukemia virus (BLV). *Bibl. Haemat. (Basel)* **43,** 235 (1976).
23. International Committee on Bovine Leukosis, Criteria for the determination of the normal and leukotic state in cattle. *J. nat. Cancer Inst.* **41,** 243 (1968).
24. R. C. Reisinger, Epizootiology of spontaneous cancer in cattle with particular reference to malignant lymphoma. *Ann. N.Y. Acad. Sci.* **108,** 855 (1963).
25. G. H. Theilen, R. D. Appleman and H. G. Wixom, Epizootiology of lymphosarcoma in California cattle. *Ann. N.Y. Acad. Sci.* **108,** 1203 (1963).
26. R. K. Anderson, D. K. Sorensen, V. Perman and J. H. Sautter, A study of environmental factors and their relationship to the occurrence of bovine lymphocytic leukemia. *Proc. U.S. Livestock Sanit. Ass.* **68,** 52 (1964).
27. G. H. Theilen, D. L. Dungworth, J. Lengyel and L. S. Rosenblatt, Bovine lymphosarcoma in California. I. Epizootiologic and hematologic aspects. *Hlth. lab. Sci.* **1,** 96 (1964).
28. G. H. Conner, J. A. LaBelle, R. F. Langham and M. Crittenden, Studies on the epidemiology of bovine leukemia. *J. nat. Cancer Inst.* **36,** 383 (1966).
29. R. K. Anderson, D. K. Sorensen, V. Perman, V. A. Dirks, M. M. Snyder and J. E. Bearman, Selected epizootiologic aspects of bovine leukemia in Minnesota (1961–1965). *Amer. J. vet. Res.* **32,** 563 (1971).
30. L. E. Baumgartener, C. Olson, J. M. Miller and M. J. van der Maaten, Survey for antibodies to leukemia (C-type) virus in cattle. *J. Amer. vet. med. Ass.* **166,** 249 (1975).
31. C. House, J. A. House and F. L. Glover, Antibodies to the glycoprotein antigen of bovine leukemia virus in the cattle population of five states. *Cornell Vet.* **67,** 510 (1977).
32. L. S. Starr and T. Young, Lymphoblastoma in dairy cattle. *Vet. Med.* **36,** 406 (1941).
33. R. R. Marshak, L. L. Coriell, W. C. Lawrence, J. E. Croshaw, H. F. Schryver, K. P. Altera and W. W. Nichols, Studies on bovine lymphosarcoma. I. Clinical aspects, pathological alterations and herd studies. *Cancer Res.* **22,** 202 (1962).
34. H. J. Bendixen, Preventive measures in cattle leukemia: leukosis enzootica bovis. *Ann. N.Y. Acad. Sci.* **108,** 1241 (1963).
35. J. E. Croshaw, D. A. Abt, R. R. Marshak, W. C. D. Hare, J. Switzer, J. Ispen and R. M. Dutcher, Pedigree studies in bovine lymphosarcoma. *Ann. N.Y. Acad. Sci.* **108,** 1193 (1963).
36. W. C. D. Hare, R. R. Marshak, D. A. Abt, R. M. Dutcher and J. E. Croshaw, Bovine lymphosarcoma. A review of studies on cattle in the eastern United States. *Canad. vet. J.* **5,** 180 (1964).
37. D. A. Abt, R. R. Marshak, H. W. Kulp and R. J. Pollock, Studies on the relationship between lymphocytosis and bovine leukosis. *Bibl. Haemat. (Basel)* **36,** 527 (1970).
38. R. H. Cypess, J. H. Waller, C. K. Redmond, R. J. Tashjian and A. I. Hurvitz, Epidemiologic and pedigree study of the occurrence of lymphosarcoma from 1953 to 1971 in a closed herd of Jersey cows. *Amer. J. Epidemiol.* **99,** 37 (1974).
39. J. M. Miller and M. J. van der Maaten, Serologic detection of bovine leukemia virus infection. *Vet. Microbiol.* **1,** 195 (1976).

40. C. Olson, L. E. Baumgartener, J. M. Miller and M. J. van der Maaten, A comparison of tests on reference serums for BLV antibody. *Vet. Microbiol.* **1,** 275 (1976).
41. J. M. Miller and M. J. van der Maaten, Use of glycoprotein antigen in the immunodiffusion test for bovine leukemia virus antibodies. *Europ. J. Cancer* **13,** 1369 (1977).
42. W. R. Harvey, *Instructions for Use of LSMLMM: Least-Squares and Maximum Likelihood General Purpose Program, 252K Mixed Model Version.* Ohio State University, Columbus (1972).
43. D. S. Falconer, *Introduction to Quantitative Genetics.* p. 165. Ronald Press, New York (1960).
44. D. S. Falconer, *Introduction to Quantitative Genetics* p. 120. Ronald Press, New York (1960).
45. R. K. Cole and J. Furth, Experimental studies on the genetics of spontaneous leukemia in mice. *Cancer Res.* **1,** 957 (1941).
46. E. C. Macdowell, J. S. Potter and M. J. Taylor, Mouse leukemia. XII. The role of genes in spontaneous cases. *Cancer Res.* **5,** 65 (1945).
47. F. Lilly, E. A. Boyse and L. J. Old, Genetic basis of susceptibility to viral leukaemogenesis. *Lancet* **ii,** 1207 (1964).
48. F. Lilly, The inheritance of susceptibility to the Gross leukemia virus in mice. *Genetics* **53,** 529 (1966).
49. S. N. Ardashnikov, The genetics of leukaemia in man. *J. Hyg. (Lond.)* **37,** 286 (1937).
50. R. C. Anderson, Familial leukemia: a report of leukemia in five siblings, with a brief review of the genetic aspects of this disease. *Amer. J. Dis. Child.* **81,** 313 (1951).
51. P. G. Rigby, R. C. Rosenlof, P. T. Pratt and H. M. Lemon, Leukemia and lymphoma. *J. Amer. med. Ass.* **197,** 25 (1966).
52. P. G. Rigby, P. T. Pratt, R. C. Rosenlof and H. M. Lemon, Genetic relationships in familial leukemia and lymphoma. *Arch. intern Med.* **121,** 67 (1968).
53. S. W. Ferguson and T. N. Lynn, Familial leukemia: a report of 4 cases of acute leukemia in 4 consecutive generations. *South. med. J. (Bgham, Ala.)* **63,** 1337 (1970).
54. F. W. Gunz, J. P. Gunz, A. M. O. Veale, C. J. Chapman and I. B. Houston, Familial leukemia: a study of 909 families. *Scand. J. Haematol.* **15,** 117 (1975).
55. W. E. Heston, The genetic aspects of human cancer. *Advanc. Cancer Res.* **23,** 1 (1976).
56. F. W. Gunz, J. P. Gunz, P. C. Vincent, M. Bergin, F. L. Johnson, H. Bashir and R. L. Kirk, Thirteen cases of leukemia in a family. *J. nat Cancer Inst.* **60,** 1243 (1978).
57. L. E. Baumgartener, J. Crowley, S. Entine, C. Olson, G. Hugoson, H.-J. Hansen and W. H. Dreher, Influence of sire on BLV infection in progeny. *Zbl. Vet-Med.* **25,** 202 (1978).
58. J. M. Miller and M. J. van der Maaten, Infectivity tests of secretions and excretions from cattle infected with bovine leukemia virus. *J. nat. Cancer Inst.* **62,** 425 (1979).
59. C. J. Wilcox, Breeding for milk production. In *Animal Agriculture.* (Edited by H. H. Cole and M. Ronning) p. 280. W. H. Freeman & Co., San Francisco (1974).