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A Preliminary Evaluation of the Danish Control Program for Enzootic Bovine Leukosis

P.Willeberg, C.E.Franti, A.Gottschau, J.Flensburg and R.Hoff-Jorgensen

Enzootic Bovine Leukosis (EBL) has in recent years attracted much attention, due mainly to economic implications of international trade regulations requiring EBL-free status when cattle are to be exported live (Reed 1980, Miller 1981, Hugoson and Wold-Troell 1982). Programs for control and eradication of EBL are therefore being considered by many countries. Such programs are based on the acquired knowledge about the causative agent (EBL-virus), the epidemiology of the infection, and testing procedures developed for diagnosing infected animals or herds, as well as on the results of control programs launched in a few countries even before the discovery of the EBL-virus in 1969.

In Denmark a program to control EBL was introduced in 1959 and its apparent effects on the rate of leukotic tumors and on the frequency of new EBL-infected herds found through the various phases of the program have been described previously (Flensburg and Streyffert 1977, Hoff-Jorgensen 1981, Flensburg 1982).

Due to the current interest in the epidemiology of the infection in general, and in EBL control programs in particular, more formal analytical studies of the data from the Danish program are presently being carried out. This paper desribes the program and its achievements, using some preliminary results of these analyses, especially for data from the hematological testing program during 1969 - 1978.

Authors' addresses: P.Willeberg, Dept. of Forensic and State Veterinary Medicine, Royal Veterinary and Agricultural University, Bulowsvej 13, DK-1870 Copenhagen V, Denmark; C.E.Franti, Dept. of Community Health, School of Medicine, University of California, Davis, California 95616; A.Gottschau, Dept. of Mathematics and Statistics, Royal Veterinary and Agricultural University, Bulowsvej 13, DK-1870 Copenhagen V, Denmark; J.Flensburg, State Veterinary Service, Frederiksgade 21, DK-1265 Copenhagen K, Denmark; R.Hoff-Jorgensen, State Veterinary Serum Laboratory, Bulowsvej 27, DK-1870 Copenhagen V, Denmark.

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THE DANISH LEUKOSIS CONTROL PROGRAM

Based on extensive epidemiological investigations during the 1950's Bendixen (1963) established that:

1. Bovine leukosis was found in two forms: sporadic and enzootic.
2. Prevalence of EBL in Denmark was low; most of the EBL was found on the eastern islands which had only 15% of the cattle.
3. The epidemiology of the disease indicated that a slow and moderately contagious virus was involved in its etiology.
4. Herd history (tumor cases) and hematological examination of the herd (lymphocytosis) were valuable diagnostic tools for identifying herds with EBL.

On these bases the veterinary authorities, actively supported by the cattle industry, introduced a leukosis control program based on the following administrative guidelines and regulations:

1. Leukosis = a permanent lymphocyte infiltration in blood (lymphocytosis) and/or tissues (tumors).
2. Sporadic leukosis = a single case of leukosis in a herd.
3. Enzootic leukosis = several cases of leukosis in a herd.
4. Enzootic leukosis could only be eradicated by slaughter of the whole herd followed by cleaning, disinfection, and restocking.

Phase 1, 1959-1968

Suspect tumors in cattle were notifiable, and material from such cases, whether found at slaughter or as clinical cases, had to be submitted for histologic examination. When leukosis was diagnosed in an animal, an epidemiological examination was carried out, including hematological examination of all adult cattle in the herd of origin and in-contact herds. Herd diagnosis and disease eradication were carried out in accordance with the administative guidelines. Herds considered EBL-positive were closed, and animals could only leave the herd directly for slaughter with a movement permit. A public indemnity based on the number and age of animals in the herd was offered in order to induce the owner to slaughter the infected herd.

Phase 2, 1969-1978

During Phase 1 it had become clear that a herd could be infected and spread the infection for many years without a clinical case of EBL occurring. Development of electronic particle counters made large numbers of white blood cell counts possible, and routine hematological herd tests of all herds in the country were initiated in 1969. The first countrywide hematological test was performed in 1969-1971, the second in 1972-1974, and the third in 1975-1978. During the third

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period an immunodiffusion test to demonstrate antibodies to EBL virus became available. The test was specific and considerably more sensitive than the hematological test. It was subsequently used to retest herds with hematological or histological evidence of leukosis.

Phase 3, 1979-1981

As of January 1979, routine immunodiffusion tests were carried out in all cattle above 24 months of age and in calves up to 6 months of age (maternal antibodies). During the 3-year period all herds on the eastern islands and all herds in which a single case of leukosis had been found since 1969 were retested in this way.

Phase 4, 1982-

Due to the low number of EBL-infected herds found during the previous phase, the program was recently changed from herd screening into monitoring of the cattle population by blood sampling at slaughter.

MATERIALS AND METHODS

As a first step in the present analysis, the existing manual files on suspected individual leukosis cases and the EBL-suspected herds, respectively, were coded and transferred to computer files. The herd file could only be accessed back to the beginning of Phase 2 of the program, and accordingly both computer files were limited to the period from 1969 through the first half of 1981.

In addition to using these two files, some analyses were performed with a merged version of the two, supplementing the tumor data with information on herd factors such as final EBL-status and herd size. Due to logistic problems in identifying certain tumor cases, not all of the tumor file records have been successfully matched with herd data at this time. Consequently, some preliminary results have to be given as proportional rates or as approximately estimated incidence rates applying the proportional distribution of the matched fraction of the cases.

Denominators for estimating incidence rates were obtained from official animal census data on herds and animals at risk by year and region, and from annual reports of the State Veterinary Service giving the numbers of animals and herds tested in the program by year and region.

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RESULTS

Among the 2184 histological submissions recorded in the "tumor file" 2130 individual animals were identified, of which 960 had leukotic tumors. Of these, 543 (57%) were diagnosed as adult leukosis , 344 (36%) as juvenile leukosis, and 73 (8%) as skin leukosis. The remaining records concerned non-leukotic and non-neoplastic conditions. Successful matching with herd data was obtained for 81% of the leukosis cases. Of the 448 matched adult leukosis records 76 (17%) were associated with EBL-positive herds. Changes in estimated incidence rates of leukotic tumors throughout Phase 2 of the program appear in Fig. 1.

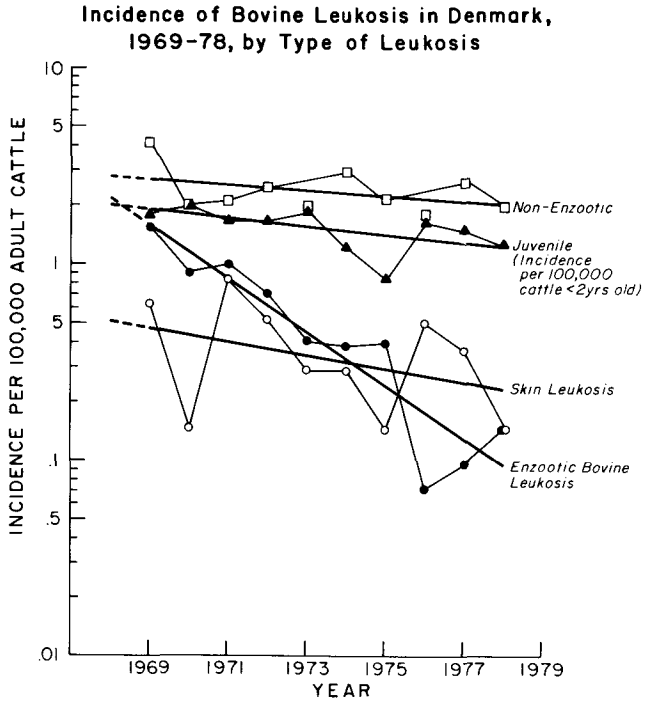

Fig. 1 Changes during Phase 2 in estimated incidence rates of different leukotic tumor types.

The herd file contains records on 3779 herds with a history of having been suspected of EBL since 1969. Of these, 502 were classified as EBL-positive. Their distribution by year and region appears in Table 1. For comparison the total numbers of EBL-herds found during Phase 1 (1959-1968) were 455 and 148 for east and west Denmark, respectively.

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Table 1. Distribution of EBL-positive herds found in Denmark by year and region; '81 covers only the first half of the year.

Region	Year 1969	'70	'71	'72	'73	'74	'75	'76	'77	'78	'79	'80	'81	All
Eastern	73	80	28	19	26	10	3	16	16	3	7	8	4	293
Western	22	35	36	26	21	10	13	8	15	8	4	5	6	209
Total	95	115	64	45	47	20	16	24	31	11	11	13	10	502

In order to evaluate the efficacy of the herd testing and screening procedures applied since 1969, taking into account the numbers of herds tested and herds at risk, the incidence rate of EBL-positive herds by year and region have been estimated and are presented in Fig.2.

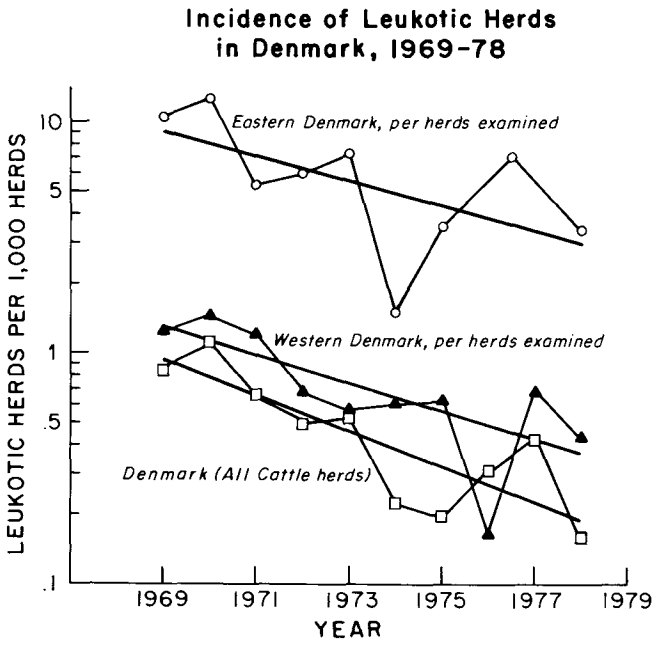

Fig. 2 Changes during Phase 2 in incidence of EBL-positive herds out of those tested by region and out of all herds in Denmark.

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As a crude estimate of the specificity of the testing and classification procedure one may use the complement of the lowest apparent incidence rate assuming that all positive herds for that reading were false positives. From Fig. 2 it may be seen that the lowest incidence rate observed was .164 per 1000 herds (western region, 1976), while the lowest estimate from the fitted regression line was about .360 per 1000 herds (western region, 1978). It may then be concluded that the specificity has been at least .999836 using the former estimate. This means that out of all 225,618 routine hematological herd tests performed during Phase 2, at most 37 were false positives. The total number of observed positive herds during this phase was 468 (Table 1), indicating that at most 8% of these may have been free from EBL. More details on these evaluative aspects will be presented elsewhere (Franti and Willeberg, in prep).

The size of the herds from which adult tumor cases originated was obtained from the matched file, and the proportional distributions of EBL- and non-EBL-associated cases are shown in Table 2.

Table 2. Distribution by herd size of 448 EBL- and non-EBL-associated adult leukotic tumor cases in the herd-matched file.

No. of cows in the herd	No. of cases EBL	No. of cases non-EBL	Proportional distr. EBL	Proportional distr. non-EBL	Odds ratio EBL vs. non-EBL
			%	%	
<15	32	79	42	21	1
15-29	21	86	28	23	.6
30-49	12	71	16	19	.4
50-	10	53	13	14	.5
Unknown	1	83	1	22	-
Total	76	372	100	99	-

Age was known for 191 of the herd-matched leukosis-affected cows; only 25 were from EBL-herds. The age distribution of the tumor cases is presented in Table 3.

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Table 3. Age distribution of 448 EBL- and non-EBL-associated adult leukotic tumor cases in the herd-matched file.

Age in years	No. of cases EBL	No. of cases non-EBL	Proportional distr. EBL	Proportional distr. non-EBL	Odds ratio EBL vs. non-EBL
			%	%	
2 - 3	11	101	14	27	1
4 - 5	9	50	12	13	1.7
6 -	5	15	7	4	3.1
Unknown	51	206	67	55	2.3
Total	76	372	100	99	-

DISCUSSION

The hematological testing procedure applied during most of the Danish leukosis control program has been characterized previously as lacking sensitivity and specificity (Flensburg and Streyffert 1977, Ferrer 1979). However, the present results, although preliminary, clearly indicate that the program did selectively reduce the incidence of EBL both at the cow level (Fig. 1) and at the herd level (Fig. 2). The fact that in 1976 in western Denmark the rate of apparently EBL-positive herds was as low as .164 per 1000 herds tested, while in subsequent years the rate went up because of the pilot testing by the serological method, indicates that hematology is considerably less sensitive in diagnosing EBL than is serology, while the specificity of the two may be considered not far from 100%. It should be stressed in this connection that in the Danish control program hematology was applied as a herd test and that repeated testing was done sequentially, both measures increasing the specificity and thereby decreasing the sensitivity of the procedure.

The implications suggested by others that in Denmark many slaughtered herds with a few hematological reactors might have been erroneously classified as EBL- positive due to a low specificity of the testing procedure is clearly refuted by the present results.However, more refined analyses are currently under way taking into account the apparent associations with age and herd size shown in Tables 2 and 3. These analyses are being performed in order to adjust specifically for the possible confounding effects caused by a general increase in average herd size in the Danish cattle population since the 1950's, younger average age of cattle in these herds, and a westward shift in the center of the cattle population of Denmark. These adjustments

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are, however, not likely to alter to any great degree the above stated general conclusions about the efficacy and efficiency of the bovine leukosis control program as conducted in Denmark.

It seems obvious therefore that the initial phases of the Danish EBL control program in which hematology was used have indeed been successful. The gains made during these phases make it feasible to believe that the ultimate eradication of EBL could occur in the subsequent phase of the program with support from the more sensitive serological methods. The recently adopted Phase 4 in which monitoring at slaughter is used as the main routine procedure may soon reveal to what extent this goal is being fulfilled.

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