Eradication of Aleutian Disease of Mink by Eliminating Positive Counterimmunoelectrophoresis Test Reactors

H. J. CHO¹* AND J. GREENFIELD²

Animal Pathology Division, Health of Animals Branch, Agriculture Canada, Animal Diseases Research Institute (Western), Lethbridge, Alberta T1J 3Z4,¹ and Veterinary Laboratory, British Columbia Department of Agriculture, Abbotsford, British Columbia V2S 4N8,² Canada

Received for publication 30 August 1977

The counterimmunoelectrophoresis test was applied on three Aleutian disease virus-infected mink ranches for the detection of specific Aleutian disease virus antibody. All mink on the ranches were tested during the pelting season and before the breeding season for 4 consecutive years. Aleutian disease has been eliminated from the three commercial mink ranches by culling out all mink that were positive for Aleutian disease virus antibody.

Aleutian disease (AD) is a common, persistent viral infection of mink which causes serious economic loss to the mink industry. AD virus (ADV) has been purified and characterized from infected mink tissues and from cell cultures and has been shown to be an autonomous parvovirus (27, 32) which is temperature sensitive in its replication (27). The pathogenesis of AD is believed to have an immunological basis (15, 18, 26). Circulating virus-antibody complexes (25) are associated with immune complex arteritis (30) and glomerulonephritis (16, 24, 28). The involvement of the host immune response has been shown by the prevention of lesions by immunosuppression with cyclophosphamide without a significant reduction in the amount of ADV in the serum (2) and by immunization with an inactivated virus vaccine followed by live virus challenge (29), which resulted in the enhancement of lesions. Effective therapeutic or prophylactic measures are not available at the present time; therefore, attempts to control this disease on a ranch basis require identification and removal of carrier animals. To achieve this objective, detection of infected animals by a method based on immunological principles is essential.

Five specific serological tests have been adapted for the accurate diagnosis of AD. These are the immunofluorescent test (28), the complement fixation test (22), the counterimmunoelectrophoresis (CIEP) test (4), the immunodiffusion test (5), and the modified CIEP test (6). The sensitivity, reproducibility, and specificity of the immunofluorescent, complement fixation, CIEP, and modified CIEP tests have been recently compared (6). Previously, the iodine agglutination test (IAT) had been extensively used in the field for the diagnosis of AD; however,

since the IAT is a nonspecific test, AD cannot be eliminated by culling out the IAT-positive mink (10). We now present data showing that by using a simple CIEP test for the detection of ADV antibody and eliminating all antibody-positive mink, AD can be eradicated from commercial mink ranches under field conditions.

MATERIALS AND METHODS

Animals. Three mink ranches were selected, and all animals were tested for the presence of ADV antibody during the pelting season and before the breeding season for four consecutive years to determine whether the CIEP test could provide a basis for eradication of AD from infected populations. All positive reactors were pelted, and only ADV antibody-negative animals were retained as breeding stock. Blood samples from 120 feral mink trapped in several areas in Northern Ontario, kindly provided by the late R. O. Ramsden, were also tested. A few mink ranches were identified in the general area within 30 miles of the feral mink trapping. In January 1974, an attempt was made to determine the prevalence of AD in several provinces in Canada. Four hundred mink ranchers were approached with a request that each submit blood samples from 20 mink. Samples were received from only 41 of these ranches, and these were also tested.

Laboratory procedures. The CIEP test was performed as described previously (4) with minor modifications. All tests were performed on glass plates (75 by 50 mm) covered with 10 ml of 1% combined medium (purified agar [Difco Laboratories, Detroit, Mich.] plus agarose [Bio-Rad Laboratories, Richmond, Calif.], 1:1) in a trisbarbital-sodium barbital buffer (pH 8.8, ionic strength 0.025) unless otherwise specified. A 0.02-ml volume of antigen was used which contained at least 8 to 16 units. The electrophoresis tank contained buffer which was double the strength of that in the agar gel. The results were interpreted 45 to 60 min after the start of electrophoresis at 200 V.

Kidney and liver were examined for histological

lesions of AD (16), and the levels of serum immunoglobulin were determined by cellulose acetate electrophoresis.

To detect virus, Aleutian-genotype mink negative for ADV antibody were inoculated with 1 ml of a 10% homogenate of liver and spleen intraperitoneally. Those that remained serologically negative for 3 months were considered to be free of virus.

RESULTS

Application of CIEP tests for the detection of ADV antibody on mink ranches. The results are summarized in Table 1. In the initial test on ranch 1 in November 1974, 849 of 995 mink in shed A were ADV antibody positive. All positive reactors were culled immediately. Final retests of the herd in February 1976 and February 1977 revealed only a single positive reactor out of 1,527 and 1,711 mink tested, respectively. On ranch 2, the initial test of old breeding stock in November 1974 revealed antibody in 592 of 1,576 animals. After culling the positive reactors, retests of the herd in February 1976 and 1977 detected no additional infected animals. On ranch 3, the initial test in November 1974 showed antibody in 51 of 1,271 mink, but again, after culling, retests of the ranch in November 1976 and March 1977 revealed no in-

fectured mink. As shown in Table 2, the prevalence of ADV infection in different sheds on the same ranch was extremely variable. On ranch 1 in November 1974, the highest prevalence (85.3%) was observed in shed A. Standard dark mink, which were considered a strain resistant to AD, were raised in this shed. A high proportion (39%) of old breeding male mink also proved to be positive reactors. Blue mink were raised in sheds C to F, but only 109 of 3,054 (3.6%) were antibody positive. Of these 109 positive reactors, 35 mink were caged singly and 74 were caged in pairs. Of the latter, in 16 cages both mink were reactors and in 42 cages only one was seropositive. On ranch 1, all the seropositive mink were culled, and the infected cages were washed with water only; on ranches 2 and 3, the infected cages were thoroughly disinfected with a 2% NaOH solution.

In the survey study of 41 commercial ranches, ADV infection was diagnosed by the CIEP test in 19 ranches. Of the 120 feral mink tested, ADV antibody was detected in 74.

Relationship of antibody titer, immunoglobulin levels, histopathology, and virus detection. Twenty-four mink which were positive in the CIEP test were sacrificed to study

TABLE 1. *Summary of serological tests for Aleutian disease on three infected mink ranches^a*

Ranch ^b	Date of bleeding	No. of tests	No. of positive reactors (%)	No. of negative reactors (%)	Remarks
1	Nov. 1974	995	849 (85.3)	146 (14.7)	Shed A
	Nov. 1974	123	48 (39.0)	75 (61.0)	Shed B old males
	Nov. 1974	3,054	109 (3.6)	2,945 (96.4)	Sheds C-F
	Jan. 1975	1,515	26 (1.7)	1,489 (98.3)	Retest sheds A-F
	Nov. 1975	583	19 (3.2)	564 (96.8)	Shed A
	Dec. 1975	2,197	2 (0.1)	2,195 (99.9)	Sheds B-F
	Feb. 1976	1,527	1 (0.06)	1,526 (99.94)	Sheds A-F retest
2	Feb. 1977	1,711	1 (0.06)	1,710 (99.94)	Sheds A-F
	Nov. 1974	1,576	592 (37.6)	984 (62.4)	Old breeding stock
	Dec. 1974	2,091	151 (7.2)	1,940 (92.8)	New selected stock
	Feb. 1975	1,876	18 (1.0)	1,858 (99.0)	Retest (prebreeding)
	Nov. 1975	420	2 (0.5)	418 (99.5)	Old breeding stock
	Dec. 1975	2,121	1 (0.05)	2,120 (99.95)	New selected stock
	Feb. 1976	2,004	0 (0)	2,004 (100)	Retest (prebreeding)
3	Nov. 1976	2,188	1 (0.04)	2,187 (99.96)	Prepelting
	Feb. 1977	2,167	0 (0)	2,167 (100)	Retest (prebreeding)
	Nov. 1974	1,271	51 (3.9)	1,220 (96.1)	Prepelting
	Feb. 1975	806	6 (0.7)	800 (99.3)	Retest (prebreeding)
	Nov. 1975	1,106	12 (1.1)	1,094 (98.9)	Prepelting
	Feb. 1976	865	2 (0.23)	863 (99.77)	Retest (prebreeding)
	Nov. 1976	1,259	0 (0)	1,259 (100)	Prepelting
	Mar. 1977	987	0 (0)	987 (100)	Retest (prebreeding)

^a Ion agar was used in 1974 tests, and the combined medium (agarose plus purified agar, 1:1) was used in the subsequent tests.

^b Color phases of mink on each ranch were as follows. Ranch 1: shed A, standard dark mink; shed B, pastel and blue mink; sheds C to F, mostly blue mink, and some pastel mink in shed F. Ranch 2: mostly standard dark mink and some pastel mink. Ranch 3: pastel mink, 60%; standard dark mink, 30%; blue mink, 10%.

TABLE 2. *Distribution of ADV-positive reactors in different sheds^a*

Shed	Total no. of mink	Color of mink	No. of reactors (%)	No. of mink per cage		
				One	Two	
				No. of positive reactors	One positive reactor (no. of cages)	Both positive reactors (no. of cages)
A	995	Standard dark	849 (85.3)			
B	123	Pastel & blue	48 (39.0)			
C	810	Blue	32 (4.0)	6	12	7
D	738	Blue	17 (2.3)	5	4	4
E	709	Blue	31 (4.4)	8	15	4
F	796	Pastel & blue	29 (3.6)	16	11	1

^a Ranch 1, November 1974 test.

the relationship between antibody titers, immunoglobulin levels, histopathological lesions, and detection of ADV by mink inoculation. As shown in Table 3, 19 of these mink had immunoglobulin levels greater than 15%, which is considered the maximum normal level (28), 18 showed histopathological lesions suggestive of AD in kidney and/or liver, and in 23, ADV was demonstrated by mink inoculation. The results of serological and histopathological studies on wild caught and ranch mink (Table 4) revealed that ADV antibody was always detected in mink with histopathological lesions, whereas seronegative mink never showed such lesions. A high percentage of the mink which were serologically positive showed no histopathological evidence of the disease, but the presence of live virus could usually be substantiated in such cases by inoculating their tissue suspension into ADV antibody-negative mink and inducing subsequent infection (Table 3).

DISCUSSION

It has been previously shown that the CIEP test is simple, rapid, accurate, and specific for the diagnosis of ADV infection (1, 5, 6). In Denmark, over the past 10 years, about 250,000 of one million breeding mink have been tested annually by IAT (13). The IAT has contributed to some extent to the reduction of AD in Chediak-Higashi syndrome mink (10). However, the IAT is nonspecific and detects only 16 to 65% of CIEP positive reactors (9, 12, 13, 18). It is obvious, therefore, that the CIEP test is the method of choice for accurate diagnosis of ADV infection on commercial mink ranches. In the present investigation, we demonstrated that by using the CIEP test for the detection of ADV antibody and by killing all mink having such antibody, AD was eradicated or nearly eliminated from the three infected mink ranches we studied. However, we are aware that attempts by other workers to eradicate the AD on com-

TABLE 3. *Relationship between antibody titers, immunoglobulin levels, histopathology, and virus detection in ranch mink*

Ranch	Color of mink	Antibody titers	% Immunoglobulin	Histology ^a	Virus ^b
2	Dark	10,000	24.2	+	+
	Dark	5,000	25.3	+	+
	Dark	500	15.3	+	+
	Dark	2,000	15.2	+	+
	Dark	2,000	17.0	+	+
	Dark	1,000	11.7	+	+
	Dark	5,000	36.1	+	+
	Dark	2,000	14.9	—	+
	Dark	5,000	20.5	+	+
	Dark	500	15.6	—	+
	Pastel	50	10.8	—	—
	Pastel	500	11.7	—	+
	Pastel	5,000	23.3	+	+
	Pastel	5,000	36.4	+	+
	Dark	5,000	17.4	—	+
	Dark	10,000	31.8	+	+
	Dark	2,000	12.9	—	+
	Dark	5,000	25.2	+	+
3	Pastel	10,000	43.0	+	+
	Pastel	5,000	35.6	+	+
	Pastel	5,000	33.4	+	+
	Pastel	5,000	25.7	+	+
	Pastel	5,000	28.2	+	+
	Pastel	10,000	33.5	+	+

^a Lesions in kidney and liver.^b By mink inoculation and detection of antibody by the CIEP test 3 months later.TABLE 4. *Serological and histological comparisons of AD in feral and ranch mink*

Type of mink	No. of mink	CIEP test		Histology	
		Result	No.	+	—
Feral	55	+	30	6	24
		—	25	0	25
Ranch	54	+	24	18	6
		—	30	0	30

mercial mink ranches using similar "test and slaughter" programs are sometimes ineffective. One reason for this discrepancy may be the

differences in virulence of ADV strains; marked differences in virulence of ADV strains have been noted previously (1). AD on a mink ranch with ADV strains of low or moderate pathogenic potential may be easier to eradicate than the disease on a ranch with a highly pathogenic ADV strain. There may also be other technical factors which make the test and slaughter program less effective. For example, use of an optimum medium is essential for the CIEP test. We have noted certain mink sera which were ADV antibody positive in a combined medium as used in this study, but antibody negative in an agarose medium. It has been found that an ADV antigen titer of 8 to 16 is required for the CIEP test; however, lower-titered antigen has often been used in studies with less effective results. Maximum precautions must be taken to prevent spread of the disease during collection of the blood samples from mink. We often observed that on ranches where the program was less than satisfactory, the farmers used toenail clippers without disinfecting them between animals. This will promote the spread of AD on the ranches.

The present trial offers strong evidence that certain naturally occurring persistent virus infections can be effectively eradicated by using a test and slaughter procedure. By launching an organized slaughtering program in the infected districts, Icelandic workers nearly succeeded in eliminating maedi and visna infection in sheep, but failed to eradicate rida (scrapie) in sheep (33, 34). Attenuated live virus vaccine for African swine fever produced persistent infection in a large percentage of vaccinated animals even without subsequent exposure to virulent virus, and thus the control program had to be discontinued (7). However, by employing a vigorous slaughter program, Cuban workers succeeded in eradicating the African swine fever outbreaks which occurred several years ago in that country (20). In the United States and Canada, the incidence and prevalence of sheep scrapie has been remarkably reduced by the vigorous slaughter program of both countries. In New Guinea, kuru is now disappearing owing to the cessation of the practice of ritual cannibalism of dead kinsmen (8).

Both vertical and horizontal transmission have been reported in ADV infection (11, 14, 23). Natural ADV infection in mink ranches seems to be more vertically than horizontally transmitted (10, 13). This conclusion has been confirmed by the following observations in this study: (i) when two mink were housed in the same cage for at least 7 months, one frequently remained seronegative although the other was seropositive (Table 2); (ii) in infected mink

ranches the distribution of AD infection was extremely variable; one shed showed over 80% seropositive mink, whereas in an adjacent shed the prevalence was less than 4%; (iii) even though ADV is very resistant to various physical and chemical treatments (reviewed in reference 3), and the infected mink shed the virus in urine, saliva, and feces (11, 19), the rate of horizontal transmission among in-contact animals or those housed in close proximity was very low. If horizontal transmission played the major role, almost all mink in the infected ranches would be expected to be infected with ADV. In ranch A (Table 1), even without disinfection of the cages, all the infected mink were eliminated with the aid of the CIEP test. In the February 1977 test, the single positive reactor could be attributed to horizontal infection. During this study it was noted that if the dam was CIEP positive, the majority of the offspring were seropositive during the subsequent pelting season. These facts suggest that on individual mink ranches, vertical transmission probably is the major method of spread of ADV infection. However, we also observed the occasional rapid spread of ADV infection in other mink ranches, which may have been infected with a highly pathogenic strain of ADV. Vertical ADV infection would appear to play an important role in maintaining and perpetuating the virus in commercial mink ranches and an even greater role in wild mink populations. Porter et al. (31) recently demonstrated experimentally that transplacental ADV infection produced a disease with markedly reduced severity of lesions. We observed that a high proportion (80%) of seropositive feral mink and about 25% of seropositive ranch mink did not show histological lesions (Table 4). This type of ADV infection has been termed nonprogressive (17) or nonpersistent (21). Nonprogressive ADV infection has been observed in 25 to 35% of naturally infected ranch mink (17). This nonprogressive ADV infection in ranch mink and particularly in wild mink populations is likely to be, on many occasions, the result of vertical transmission of the disease. The mink infected in this fashion is asymptomatic and lives long enough to reproduce and thus could transmit infection to seronegative mink horizontally and, more importantly, from one generation to the next vertically. The present study has clearly shown that such infection can be effectively detected by a simple serological test. Since the mode of transmission of AD seems more vertical than horizontal in nature, the culling of CIEP seropositive mink together with appropriate disinfection and decontamination procedures should result in the control and eradication of infection on individual ranches.

ACKNOWLEDGMENTS

We wish to record our appreciation of the late R. O. Ramsden for providing the sera and the histological slides of feral mink, K. Jericho for conducting the histopathological studies on feral and ranch mink, S. E. Magwood for his interest and encouragement during this investigation, and L. Delgado and E. Tanaka for their excellent technical assistance. The cooperation of participating mink ranchers is also gratefully acknowledged.

LITERATURE CITED

- Bloom, M. E., R. E. Race, W. J. Hadlow, and B. Chesebro. 1975. Aleutian disease of mink: the antibody response of sapphire and pastel mink to Aleutian disease virus. *J. Immunol.* 115:1034-1037.
- Cheema, A., J. B. Henson, and J. R. Gorham. 1972. Aleutian disease of mink. Prevention of lesions by immunosuppression. *Am. J. Pathol.* 66:543-552.
- Cho, H. J. 1976. Purification and structure of Aleutian disease virus, p. 159-174. *In* R. H. Kimberlin (ed.), *Slow virus diseases of animals and man*. Elsevier/North Holland, Amsterdam.
- Cho, H. J., and D. G. Ingram. 1972. Antigen and antibody in Aleutian disease in mink. I. Precipitation reaction by agar-gel electrophoresis. *J. Immunol.* 108:555-557.
- Cho, H. J., and D. G. Ingram. 1973. Antigen and antibody in Aleutian disease in mink. II. The reaction of antibody with the Aleutian disease agent using immunodiffusion and immunoelectrophoresis. *Can. J. Comp. Med.* 37:217-223.
- Crawford, T. B., T. C. McGuire, D. D. Porter, and H. J. Cho. 1977. A comparative study of detection methods for Aleutian disease viral antibody. *J. Immunol.* 118:1249-1251.
- De Boer, C. J., I. C. Pan, and W. R. Hess. 1972. Immunology of African swine fever. *J. Am. Vet. Med. Assoc.* 160:528-532.
- Gajdusek, D. C. 1977. Unconventional viruses and the origin and disappearance of kuru. *Science* 197:943-960.
- Gierloff, B., and A. Thordal-Christensen. 1975. Aleutiansyge hos mink. Studie over infektionens forekomst og betyding ved pavising af specifikt antistof v.h.a. modstrøms elektroforese. *Dan. Vet. Tidsskr.* 58:589-595.
- Gorham, J. R., J. B. Henson, T. B. Crawford, and G. A. Padgett. 1976. The epizootiology of Aleutian disease, p. 135-158. *In* R. H. Kimberlin (ed.), *Slow virus diseases of animals and man*. Elsevier/North Holland, Amsterdam.
- Gorham, J. R., R. W. Leader, and J. B. Henson. 1964. The experimental transmission of a virus causing hypergammaglobulinemia in mink: sources and modes of infection. *J. Infect. Dis.* 114:341-345.
- Greenfield, J., R. Walton, and K. R. Macdonald. 1973. Detection of Aleutian disease in mink: serum plate agglutination using iodine compared with precipitation by agar-gel electrophoresis. *Res. Vet. Sci.* 15:381-383.
- Hansen, M. 1976. An investigation by means of counter-immunoelectrophoresis for plasmacytosis in mink in some Danish farms. *In* Proceedings First International Scientific Congress in Fur Animal Production. Helsinki, Finland.
- Henson, J. B., J. R. Gorham, and R. W. Leader. 1963. The familial occurrence of hypergammaglobulinemia in mink. *Tex. Rep. Biol. Med.* 21:37-42.
- Henson, J. B., J. R. Gorham, T. C. McGuire, and T. B. Crawford. 1976. Pathology and pathogenesis of Aleutian disease, p. 175-205. *In* R. H. Kimberlin (ed.), *Slow virus diseases of animals and man*. Elsevier/North Holland, Amsterdam.
- Henson, J. B., J. R. Gorham, C. A. Padgett, and W. C. Davis. 1969. Pathogenesis of the glomerular lesions in Aleutian disease of mink. Immunofluorescent studies. *Arch. Pathol.* 8:21-28.
- Ingram, D. G. 1976. Aleutian disease, p. 8-9. *In* Canada Mink Breeders Association Educational Bulletin No. 53.
- Ingram, D. G., and H. J. Cho. 1974. Aleutian disease of mink: virology, immunology and pathogenesis. *J. Rheumatol.* 1:74-92.
- Kenyon, A. J., C. F. Helmboldt, and S. W. Nielsen. 1963. Experimental transmission of Aleutian disease with urine. *Am. J. Vet. Res.* 24:1066-1067.
- Kouba, V., and J. Straka. 1972. African swine fever in Cuba and its successful eradication. *Veterinarstvi* 7:298-300.
- Larsen, A. E., and D. D. Porter. 1975. Pathogenesis of Aleutian disease of mink: identification of nonpersistent infection. *Infect. Immun.* 11:92-94.
- McGuire, T. C., T. B. Crawford, J. B. Henson, and J. R. Gorham. 1971. Aleutian disease of mink: detection of large quantities of complement fixing antibody to viral antigen. *J. Immunol.* 107:1481-1482.
- Padgett, G. A., J. R. Gorham, and J. B. Henson. 1967. Epizootologic studies of Aleutian disease. I. Transplacental transmission of the virus. *J. Infect. Dis.* 117:35-38.
- Pan, I. C., K. S. Tsai, and L. Karstad. 1970. Glomerulonephritis in Aleutian disease of mink. Histological and immunofluorescence studies. *J. Pathol.* 101:119-127.
- Porter, D. D., and A. E. Larsen. 1967. Aleutian disease of mink: infectious virus-antibody complexes in the serum. *Proc. Soc. Exp. Med.* 126:680-682.
- Porter, D. D., and A. E. Larsen. 1974. Aleutian disease of mink. *Prog. Med. Virol.* 18:32-47.
- Porter, D. D., A. E. Larsen, N. A. Cox, H. G. Porter, and S. C. Suffin. 1977. Isolation of Aleutian disease virus of mink in cell culture. *Intervirology* 8:129-144.
- Porter, D. D., A. E. Larsen, and H. G. Porter. 1969. The pathogenesis of Aleutian disease of mink. I. *In vivo* replication and the host antibody response to viral antigen. *J. Exp. Med.* 130:575-589.
- Porter, D. D., A. E. Larsen, and H. G. Porter. 1972. The pathogenesis of Aleutian disease of mink. II. Enhancement of tissue lesions following the administration of a killed virus vaccine or passive antibody. *J. Immunol.* 109:1-7.
- Porter, D. D., A. E. Larsen, and H. G. Porter. 1973. The pathogenesis of Aleutian disease of mink. III. Immune complex arteritis. *Am. J. Pathol.* 71:331-334.
- Porter, D. D., A. E. Larsen, and H. G. Porter. 1977. Reduced severity of lesions in mink infected transplacentally with Aleutian disease virus. *J. Immunol.* 119:872-876.
- Shahrabadi, M. S., H. J. Cho, and R. G. Marusyk. 1977. Characterization of the protein and nucleic acid of Aleutian disease virus. *J. Virol.* 23:353-362.
- Sigurdsson, B. 1954. Maedi, a slow progressive pneumonia of sheep: an epizootological and a pathological study. *Br. Vet. J.* 110:255-270.
- Sigurdsson, B. 1954. Rida, a chronic encephalitis of sheep. *Br. Vet. J.* 110:341-354.