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The Transmission of Distemper Among Ferrets and Mink

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Distemper is a common virus disease of certain carnivores which is characterized by febrile signs, nasal and conjunctival exudate, and occasionally by signs indicating central nervous system damage. Its rapid dissemination in a susceptible population has long been recognized. Fragmentary information suggests that the virus may be transmitted by direct and indirect contact and through the air. Investigators have reported that transmission may be accomplished by other mechanisms.[1-4] However, there are no definitive researches elucidating these means of transmission. Of particular significance, the infectious period of distemper-virus (DV) infection in animals is not known. Such knowledge is of the greatest importance in planning measures for the control of the malady. The studies to be reported in this paper form part of an investigation of the natural history of the distemper virus among ferrets and mink.

This is a coöperative investigation between the Department of Veterinary Science, University of Wisconsin and the Pathological Division, Bureau of Animal Industry, U.S.D.A.

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REVIEW OF LITERATURE

Early in the nineteenth century, Jenner[5] provided the first accurate description on how extremely contagious the distemper virus can be. He wrote, "It is as contagious among dogs as the small-pox, measles, or scarlet fever among the human species; and the contagious miasmata, like those rising from the diseases just mentioned, retain their infectious properties a long time after separation from the distempered animal."

In 1851, Blaine[6] recognized the extreme transmissibility of the disease among dogs. He reported that "even being exposed to the air impregnated with the exhalations from a distempered dog for a few minutes is sufficient for the purpose."

According to Kirk[7] early workers demonstrated that the nasal exudate was infective. These individuals believed that a vaccine could be prepared from the nasal discharges of infected dogs. The method was eventually abandoned because a large number of the dogs contracted distemper and many died.

Some of the first investigations relative to the transmission of the disease are cited by Hutyra, Marek, and Manninger[1] They state, "The infective nature of the disease was proved in the middle of the last century by numerous experiments in which the nasal secretion (Renner and Karle, and others) the saliva (Venuta) and the contents of cutaneous pustules (Trasbot) of infected animals were found to convey infection to young dogs." Hutyra *et al.* did not supply references to the original communications.

In 1905, Carré[8] reported that the malady was caused by a virus. His experiments provided the first definitive evidence of the infectious nature of the nasal exudate. The inoculation of nasal exudate from an infected dog caused the death of puppies. When filtered nasal secretion collected from dogs in the early stages of the disease was injected into older dogs, distemper signs were noticed. If the nasal exudate was obtained from dogs at a later time during the

disease and inoculated into other dogs, no signs of distemper were observed. Infection occurred when healthy dogs were placed with experimentally infected animals.

Kirk,[5-7] in 1922, provided an excellent review of the early literature on the malady. He believed that the most important source of infection was direct contact with a diseased animal but that transmission could be accomplished indirectly through contaminated kennels and feeding utensils. Kirk thought that the dog was capable of transmitting distemper as long as it showed signs of the disease and during convalescence.

Dunkin and Laidlaw[9] were the first workers to supply critical evidence of transmission of DV under adequately controlled conditions. In addition to artificial procedures, they demonstrated the transmissibility of the virus in natural situations. They noticed that contact between a sick ferret and an unexposed ferret for a short time was "almost certain" to result in infection of the normal individual. Apparently, this procedure was employed to maintain one of their strains of virus. It was suggested that transmission was more likely if ferrets in the earlier stages of the disease were placed with susceptible animals. They reported that spread to unexposed ferrets was also accomplished in the later stages of the disease. These workers were able to show that pens from which moribund ferrets had been removed remained infective for susceptible ferrets for some hours. Because of this latter observation, they were convinced that the secretions and excretions of sick ferrets were infectious. In other experiments, transmission of the virus through the air for short distances was demonstrated. Inoculated ferrets were placed approximately 2 ft. away from unexposed animals. Ten days after the inoculated ferrets first showed signs of distemper, the previously unexposed individuals developed the disease. Other possible sources of infection, *i.e.*, infection by food, ectoparasites, and flies, were eliminated. Transmission of DV, without actual contact of the animals, from dog to ferret, and the converse, was described. Significantly, the transfer of virus between infected and control animals in their experimental house was so frequent that they abandoned trials with dogs in confined places and used outside kennels and runs. They separated each run and kennel a distance of 15 to 20 yd. from the closest neighboring unit. It was suggested that there may have been a dilution effect of the air which prevented the spread of DV to uninoculated controls. In one instance, it was thought that the wind may have carried the virus approximately 15 yd.

Green[10] demonstrated the presence of DV in nasal washings. The nasal washings from infected foxes were diluted and filtered through Berkefeld N. filters; healthy animals were inoculated and distemper was produced in a few individuals.

Laidlaw[11] observed that the feeding of experimental animals with tissues from distemper animals gave variable results. Ferrets did not become infected after chewing and swallowing tissue containing millions of infective doses as determined by inoculation in ferrets.

It is difficult to evaluate the transmission trials of Kantorowicz.[3] There is a possibility that he was dealing with an agent other than distemper, or that he may have been working with a mixed infection.

Shaw[12] rubbed skin lesions from infected mink into a scarified area of skin of normal adult mink. He did not effect transmission of DV in this instance.

According to Rothel,[4] distemper was transmitted in some cases by injecting urine of infected animals or eye material from a dog affected with keratitis. It was also claimed that virus was transmitted from cats to dogs and vice versa. Present knowledge suggests that he was dealing with a concurrent infection.

De Monbreun[2] indicated that transmission was possible by feeding tissues containing virus. He fed milk that contained a suspension of virus-infected liver to 2 puppies. Both animals succumbed after the feeding. In another trial, virus-containing liver was rubbed into a scarified area of skin of a puppy. During the ensuing observation period, the animal did not show definite signs of distemper. Approximately two months after the skin inoculation, it was challenged with an intravenous inoculation of virus and the "animal proved to be immune." One young dog was inoculated intraperitoneally with urine collected from another infected puppy at necropsy. The animal died eighteen days later. In his transmission experiment, De Monbreun used as source virus the spleen of a dog which died after an acute attack. Microscopic examination of tissues from this dog revealed intranuclear inclusions in the hepatic cells. These clinical and pathological findings suggested infectious hepatitis, but since sections of the lungs of this dog showed inclusions in bronchial and alveolar epithelium it is probable that the dog had a concurrent DV infection. The possibility of a dual infection has been pointed out previously by Kriesel[13] and Green and Evans.[14]

Gorham and Boe[15] found that the nasal exudate of experimentally infected ferrets contained virus in infectious quantities on the fifth, sixth, eighth, tenth, eleventh, and thirteenth days after subcutaneous exposure. Distemper virus was not demonstrated in the exudate collected on the second and fourth day of the experiment.

Apparently, Morris and Coburn[16] were the first workers to successfully infect animals with DV by means of an aërosol. Ferrets were exposed for one-minute intervals with a virus suspension prepared from tissues of distemper-infected ferrets.

Baker and Gorham[17] placed susceptible adult mink in wire pens adjacent to wire pens contain-

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ing infected ferrets. Although none of the mink showed definitive signs of the disease, serums of all had demonstrable neutralizing antibodies forty-seven days after exposure. They believed that the virus had diffused through the air but they could not entirely eliminate the possibility of spread by feeding and watering.

Dräger and Schindler[18] described spontaneous distemper infection among uninoculated dogs. The dogs were housed in boxes which were open at the top and had walls 2 m. high. Whether the virus had spread from an artificially injected dog by care-taking procedures or through the air was not ascertained.

Recently, Martin[19] pointed out that parenteral administration of DV was unsatisfactory in experimental procedures because it was not the natural mode of exposure. In order to demonstrate that the respiratory tract was the natural portal of entry, he confined ferrets to a box and introduced an aërosol of a suspension of infected-ferret spleen through a small opening. Four ferrets were placed in the box for fifteen minutes and another ferret was exposed for ten minutes on a later date. All ferrets became ill and succumbed.

MATERIALS AND METHODS

Animals.—The ferrets and mink used in these experiments were reared in semi-isolation at the University of Wisconsin fur-animal farm. The stock herds had never been vaccinated against distemper nor had they experienced an outbreak of distemper. The animals were maintained on a ration composed of cereals and horse meat. All of the mink were approximately 10 weeks of age at the beginning of the experiments. The ferrets ranged in age from 10 weeks to 11 months.

Isolation Facilities.—After inoculation of DV for passage purpose or experimental exposure for detecting virus, the animals were confined to individual portable wire pen units* which could be easily disinfected and transported. The single pens were located roughly 20 yd. apart in an enclosure surrounded by a high fence. Because of the constant danger of transferring infection among pens, the ferrets used to test for the presence of virus were inoculated on the same day in groups of 20. Four uninoculated controls were provided for each group. If any ferret showed signs of distemper sixteen or more days following experimental exposure, the observation was disregarded and that part of the experiment was repeated. Such occurrences were believed to be the result of accidental or secondary infection since by using large numbers of ferrets it was found that the incubation period of the Delavan strain of DV rarely exceeded thirteen days.

Virus.—The single strain of distemper virus used throughout the experiments was obtained from a natural outbreak of distemper which occurred on a large mink ranch near Delavan, Wis., in June, 1951. Isolation was accomplished by inoculation of ferrets and young mink subcutaneously with spleen material from field cases. This virus was typical of those commonly encountered on mink ranches. The virus was designated as the Delavan strain. The incubation period in ferrets to the development of initial eye signs was eleven to thirteen days. Ferrets rarely died in less than twenty days after inoculation. Because of this long course in ferrets, the strain resembles the Glasgow S123 strain[20] and the so-called "hard pad" virus.[21] With the classical Laidlaw-Dunkin strain[9] and the Green strain,[22] death of the ferret usually occurs at eleven to thirteen days.

Neurotropic signs were common after subcutaneous inoculation of mink and natural exposure of ferrets to the Delavan strain of virus. Thirty per cent of the mink that survived the catarrhal phase of the disease succumbed to neurotropic distemper. The slower course of the disease may have allowed invasion and a degree of localization in nerve tissue which does not ordinarily occur with the strains which are fatal within thirteen days.

The incubation period of the disease in the mink was approximately the same as in the ferret. It was somewhat dependent on the quantity of virus introduced. In ferrets and young mink, the case fatality rate from artificial exposure was 100 per cent and 86 per cent, respectively. Mink or ferret spleens containing the Delavan strain of DV were stored *in toto* or were finely ground in Ten Broeck grinders and stored as a 10 per cent suspension in 1 per cent tryptone broth. Occasionally 1 ml. of a 10^{-1} dilution of splenic tissue was inoculated into ferrets to insure maintenance of the virus.

Criteria for a Positive Test for Virus in Ferret.—The appearance of typical conjunctivitis, nasal exudation, and dermatitis, and, in doubtful cases, the demonstration of DV inclusion bodies in the epithelium of the urinary bladder were considered necessary for a positive test. The Shorr method[23] was used for staining the bladders. The animals were observed daily for signs of distemper. Ferrets that did not become infected during a trial were isolated for a period of not less than forty days and used for other purposes. Laidlaw and Dunkin[24] and Baker and co-workers[25] have shown that the administration of subinfective doses of virulent DV does not induce a significant degree of resistance in ferrets.

Preparation and Storage of Stock Virus and Infective Materials.—Penicillin and streptomycin were added to a final concentration of 500 units of each per milliliter to suppress bacteria in preparations of stock virus, nasal and conjunctival exudate, skin scruf, feces, urine, flies, and gelfoam pads. The inoculum was held at room temperature for forty-five minutes before injection. Inoculations of infective material to demonstrate the presence of DV were occa-

*In some of the experiments colony-type pens and other single pen unit arrangements were used. These will be described under the specific experiment.

sionally done immediately after collection. In the majority of the trials, however, the inoculations could not be conveniently accomplished within a short interval of time and the material was stored. This was done by placing solid material or liquids in glass tubes and storing them at −20 C.

Nasal and Conjunctival Exudate.—The technique of Burnet and Bull[26] was followed in collecting nasal exudate from ferrets and mink except that anesthesia was omitted. The animal's muzzle was submerged in 10 ml. of 1 per cent tryptone broth (5 C.). Repeated immersion of the muzzle in the broth caused the animal to expire forcibly into the fluid. One milliliter of broth was collected from each of the several Petri dishes and pooled to constitute the sample.

Small quantities (0.25 ml.) of 1 per cent chilled tryptone broth were introduced into the conjunctival sac to permit the exudate to be aspirated.

Skin Scurf.—Mink and ferrets affected with distemper often have a brownish granular material near the hair roots on the surface of the skin. Although it may contain blood elements, it is termed "scurf." This scurf and any hair that adhered to it was rubbed off the animal onto a sterile paper. The material was placed in a mortar with tryptone broth and triturated to make an approximate 10 per cent suspension. The sample was centrifuged for twenty minutes at 6,000 r.p.m. in an angle head centrifuge prior to final processing for inoculation.

Feces.—Freshly voided fecal specimens as well as colon contents obtained at necropsy were collected and frozen. The fecal samples were thawed at room temperature. The several steps in the preparation were: (1) Twenty-five gram of feces were ground in a mortar with sufficient chilled tryptone broth to make a 10 per cent suspension; (2) the suspension was allowed to sediment for thirty minutes at 5 C.; (3) the fluid was decanted and centrifuged in an angle head centrifuge for thirty minutes at 6,000 r.p.m.

Urine.—Because of the intractable nature of mink and ferrets, no attempt was made to obtain urine with a catheter. Distemper-infected ferrets were destroyed and the urine obtained at necropsy.

Flies.—Screened fly traps were used to collect flies. After collection, the traps were placed in the rancher's coldroom (−10 C.) until the flies were killed. The flies were identified, pooled, put in insulated containers, and taken to the laboratory. The composites from both ranches consisting almost entirely of *Musca domestica* and Calliphora sp. were held at −20 C. for approximately one month before processing. Approximately 30 Gm. of flies were ground in a mortar with 1 per cent chilled tryptone broth to make a 10 per cent suspension. The suspension was allowed to sediment for thirty minutes at 5 C.; the supernatant liquid was centrifuged for thirty minutes at 6,000 r.p.m. in an angle head centrifuge.

Mink-Handling Mitts.—In order to determine whether DV was present on mitts, the following plan of investigation was instituted: (1) Infected mink or ferrets were handled in the usual manner; (2) the mitts were held at room temperature (21 C.); (3) the area of the mitt (usually the thumb) that appeared damp from saliva, nasal or conjunctival exudate, was selected; (4) 1 per cent chilled tryptone broth was allowed to drain over that portion of the glove, and the washings collected.

Collection of Air Surrounding Infected Animals.—Samples of air were obtained through the use of a collection apparatus which consisted of a Cenco vacuum pump, rubber tubing, and a funnel.[27] A small glass tube and membrane holder were inserted in the vacuum line. The critical aperture of the glass tube* allowed 4.97 liters of air to pass through the membrane holder during each minute the pump was in operation. A small disc or membrane of dry gelfoam** was used as a filter for the collection of virus. After operation for a given time, the gelfoam filter was placed in 10 ml. of chilled tryptone broth and disintegrated with a glass rod. The resulting suspension was centrifuged lightly and the supernatant fluid used for inoculum.

Artificially Created Aërosol.—The equipment consisted of a Cenco pressure pump which provided a constant air pressure, a gauge, a De Vilbiss type 40 nebulizer,† and a funnel (Sinha *et al.*).[28] The method used was as follows: (1) The chamber was filled with a spleen suspension containing the virus; (2) the ferret's muzzle was placed in the funnel; (3) the pump was turned on for the desired interval time; (4) the ferret was removed and placed in an isolation pen.

Experimental Plans and Results

EXPERIMENT 1—SECRETA AND EXCRETA

Nasal Exudate.—While it has been shown that inoculation of susceptible animals with the nasal exudate of distemper-infected carnivores will produce distemper, it has not been determined when the virus first appears in the secretions of the inoculated animals. Similarly, it is not known how long following the disappearance of clinical signs the virus may persist in the exudate.

Attempts to determine the infectious period as related to clinical signs included the following steps: After inoculation of young mink with the Delavan strain of distemper virus, nasal fluids were collected beginning

*Prepared by Chemical Corps Biological Laboratory, Camp Detrick, Md.

**Supplied by the Upjohn Company, Kalamazoo, Michigan.

†Described by Rosebury[29] and manufactured by the De Vilbiss Company, Toledo, Ohio.

on the third day following inoculation. The pooled secretions of each day were tested for the presence of virus by inoculating them into susceptible ferrets.

Trial A.—Ten mink kittens representing both sexes were inoculated by the subcutaneous route with an infected ferret spleen suspension on Aug. 2, 1951. Pairs of mink received 1 ml. of each of the following dilutions: 2×10^{-1}, 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}.

The clinical signs recorded in figure 1 are on a group rather than on an individual basis. Watery secretion from the eyes and a swelling of the lids appeared in 1 mink on the eleventh day after inoculation; by the sixteenth day all of the 10 mink were showing signs of distemper. As the disease progressed, the exudate became purulent and accumulated as a dry, brown, granular mass which pasted the lids and partly occluded the eyes and the nose of some of the mink. These signs were often accompanied by a swelling of the foot pads. On day twenty-one, twenty-four, twenty-five, thirty, and thirty-one a mink succumbed; none of the animals showed nervous signs other than terminal convulsions. One death was recorded on the thirty-fourth and thirty-fifth days. Both of these latter animals exhibited neurotropic signs in addition to the previously described manifestations. Clinical evidence of distemper was absent among the survivors on the thirty- sixth day following inoculation. Two apparently healthy mink succumbed to neurotropic distemper on the sixty-eighth and eighty-ninth days. Characteristic nervous signs included hyperexcitability, clonic tremors, intermittent convulsions, semicoma, coma and, finally, death. The surviving mink was destroyed on the one-hundredth day.

The results of the search for virus in the nasal exudate of these mink are included in figure 1. The first specimens were collected and pooled on the third day and on subsequent days after inoculation of the 10 mink. The pooled specimens were inoculated into 2 ferrets subcutaneously, with each ferret receiving approximately 5 ml. of the nasal exudate-broth mixture. In all instances, both of the test ferrets died of distemper.

The data indicate that the Delavan strain of DV was first present in the nasal exudate on the fifth day postinoculation and persisted until the fifty-first day. Virus was demonstrable six days before the manifestation of clinical signs and for at least fifteen days following the disappearance of signs.

Since 2 mink succumbed to neurotropic distemper subsequent to day fifty-one, the data suggest that mink displaying this manifestation late in the course of disease may be incapable of transmitting the virus by means of the nasal exudate. Tissues from these animals were not tested for the presence of virus.

The small number of animals used in this trial did not permit evaluation of the effect of dosage on the length of the incubation period. However, the first 3 mink to show signs of the disease did receive the lowest dilutions of virus.

Trial B.—As a part of another investigation, 12 young male and female mink were divided into four groups of 3 mink each. One milliliter each of 2×10^{-1}, 10^{-1}, 10^{-2}, and 10^{-3} dilutions was given to each individual in the respective groups on July 17, 1952.

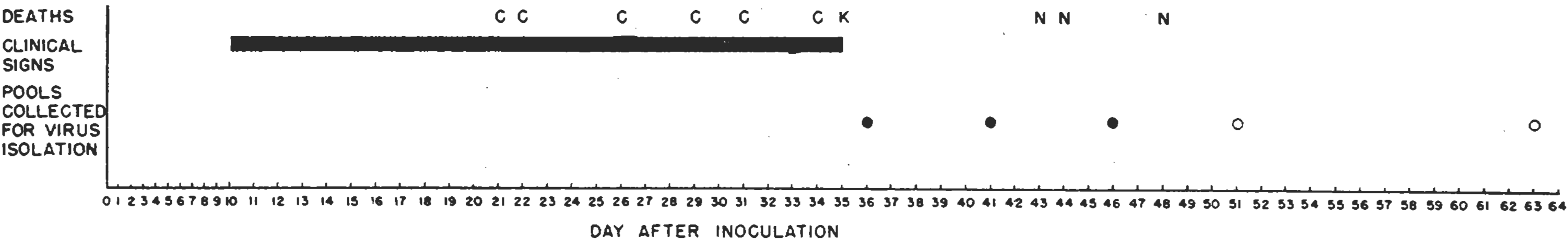
The observations are recorded in the same manner as in trial A and are summarized in figure 2. The first signs of distemper appeared on the tenth day postinoculation and continued until the thirty-fifth day. Mink succumbed to a catarrhal form of the disease on days twenty-one, twenty-two, twenty-six, twenty-nine, thirty-one, and thirty-four. Deaths following a neurotropic syndrome occurred on days forty-three, forty-four, and forty-eight after inoculation. Samples were not collected early in this experiment, but pools collected thirty-six, forty-one, forty-six, fifty-one, and sixty-three days postinoculations were prepared and each inoculated into 2 test ferrets.

As in trial A, all ferrets succumbed where a single animal of a group developed signs. These results revealed that virus was present in the nasal exudate up to the forty-sixth day but not after that date.

After the evanescence of skin and mucous membrane lesions, 3 of the 12 mink died of nervous distemper. The 2 surviving mink were destroyed on the seventieth day of the trial. The mink that received the lowest dilutions of virus (2×10^{-1} and 10^{-1}) showed eye signs before those inoculated with the higher dilutions. It was estimated that the difference was not greater than thirty-six hours.

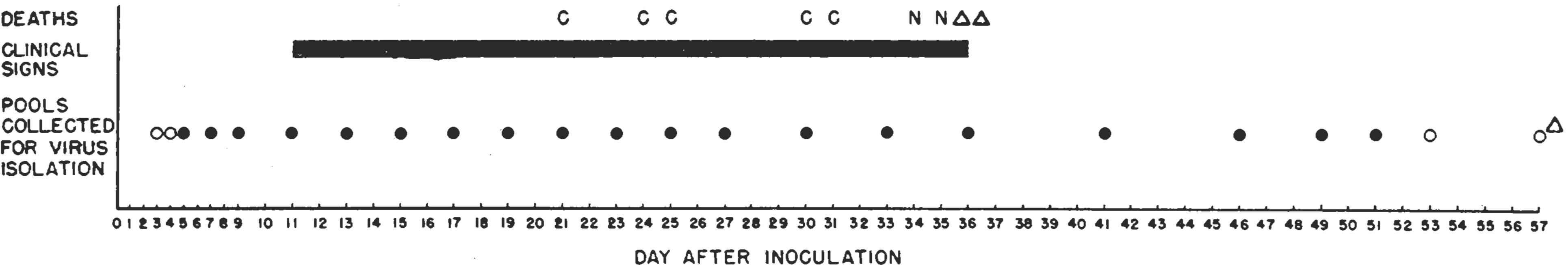
Trial C.—The data summarized in figure 3 were gathered from several other investigations where it was desired to test for virus in the nasal exudate. Each specimen was inoculated into 2 susceptible ferrets for the detection of DV.

Virus was not demonstrated in the sample collected from a single ferret five days after inoculation (isolation attempt 1). This was the only instance in which virus was not demonstrated on the fifth day postinoculation of either ferrets or mink. In isolation attempts 2 and 3, the samples were pooled from 2 or more individual animals. One ferret was used for a source of exudate in isolation attempt 4. Gorham and Boe[15] had previously been successful in detecting DV in the nasal exudate of ferrets. They employed the same technique for collecting the



●—DV demonstrated in pooled specimen; ○—DV not demonstrated in pooled specimen; C—mink dying during catarrhal stage; N—mink dying of neurotropic distemper; △—virus pools were collected on subsequent days as indicated in text; △△—fate of remaining mink given in text.

Fig. 1—Results of tests for distemper virus in the nasal exudate of 10 young mink during the course of the disease.



●—DV demonstrated in pooled specimen; ○—DV not demonstrated in pooled specimen; C—mink dying during catarrhal stage; N—mink dying of neurotropic distemper; K—killed and eaten by its pen mates.

Fig. 2—Results of tests for distemper virus in the nasal exudate of 12 young mink during the course of the disease.

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exudate as was used in this work. Their results are included in figure 3. Since the virus has been demonstrated in conjunctival washings sixteen days following inoculation, it is very probable that the infectious period of nasal exudate also extended to that post-inoculation date.

Conjunctival Exudate.—In an effort to demonstrate DV in conjunctival exudate, material was collected from 2 mink and a ferret that were showing signs of distemper. It may be noticed in table 1 that virus was present in all of the collected specimens.

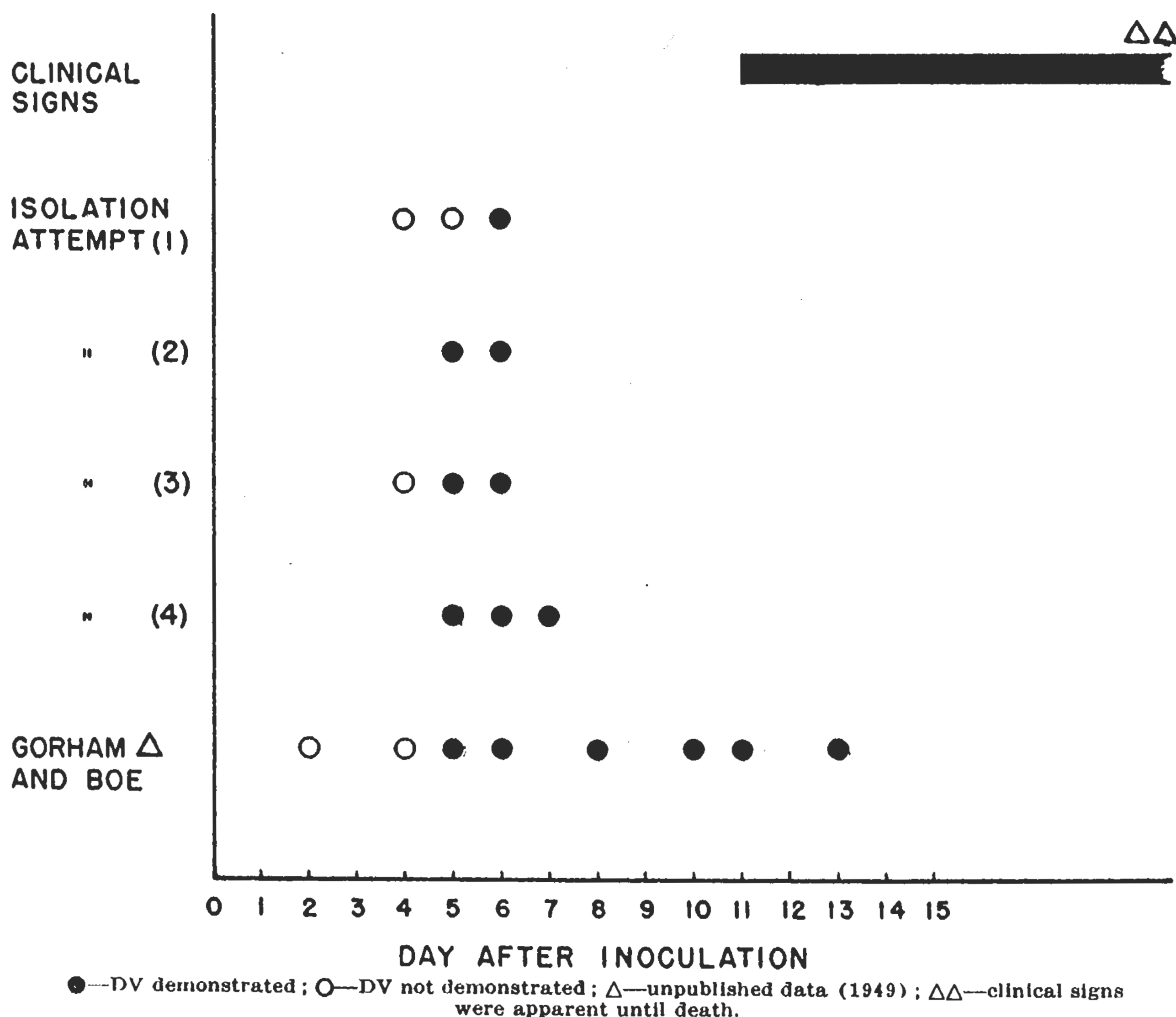
Skin Scurf.—The available literature does not record attempts to isolate DV from the granular scurf of DV-infected animals.

Approximately 5 Gm. of material was scraped from an infected moribund mink twenty-five days after inoculation. Five-milliliter quantities of inoculum were injected subcutaneously into each of 2 ferrets, both of which succumbed to distemper.

Scurf collected from the foot pads of a mink in the advanced stages of distemper was prepared and administered subcutaneously in 5-ml. doses to each of 2 test ferrets. Virus was not detected in this specimen. Later tests showed the ferrets to be susceptible to distemper.

The scrotum of an infected male ferret provided scurf for virus testing. Five milliliters of a suspension was injected into a single ferret. After a twelve-day incubation this animal developed signs of distemper and succumbed.

Feces.—The feces of infected animals have been suspected of being a source of the virus and a factor in dissemination of distemper. Hagan and Bruner[30] state "The urine and fecal material contain virus and are capable of transmitting the disease." They did not refer specifically to the finding. Since the virus has been detected in the nasal exudate, it is logical to assume that virus may be swallowed with the secretions of the upper respiratory tract, pass through the gut, and appear in the stool. Laidlaw and Dunkin[23] have reported that, in acute distemper of dogs, the Peyer's patches and mesenteric lymph nodes are usually enlarged



●—DV demonstrated; ○—DV not demonstrated; Δ—unpublished data (1949); ΔΔ—clinical signs were apparent until death.

Fig. 3—Results of tests for distemper virus in the nasal exudate of ferrets during the course of the disease.

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and edematous. They collected nodes from 3 dogs and found " the virus content to be very high; a millionth of a gram proved to be infective on two occasions." Furthermore, Green and Evans[14] have demonstrated distemper inclusions in the mucosa of the stomach, small intestine, and colon of distemper-susceptible animals.

TABLE 1—Subcutaneous Inoculation of Ferrets with Conjunctival Exudate from Infected Animals

Source of exudate	Day after inoculation specimen collected	Dose per ferret (ml.)	Results*
Mink	21	1.0	2/2
Mink	30	0.5	2/2
Ferret	16	0.9	2/2

*Numerator indicates number of ferrets that died; denominator, the number inoculated.

Freshly voided samples were assembled from the 10 DV-infected mink described in experiment 1, trial A. The feces of the thirteenth, fifteenth, seventeenth, and nineteenth days after inoculation were pooled. Inasmuch as virus was isolated from the nasal exudate composites on these days, that investigation serves as a control for this trial. Inoculation of 2-ml. quantities into 2 ferrets failed to demonstrate virus in the four fecal pools.

The colon contents of 2 infected ferrets killed *in extremis* were pooled and prepared. Four ferrets were injected with 2-ml. of the suspension. None became infected, and all were found later to be susceptible to DV.

Urine.—The epithelium of the urinary bladder and renal pelvis is generally considered to be the most satisfactory tissue of distemper-infected animals for the demonstration of inclusion bodies. A test for virus in bladder tissue was carried out with urinary bladders removed from 5 ferrets destroyed at various stages of the disease. A 20 per cent suspension of bladder tissue in tryptone broth was treated with antibiotics and inoculated subcutaneously in 1-ml. quantities into 2 ferrets. Both ferrets developed typical signs and succumbed to distemper.

Since inclusion bodies are indicative of virus activity and inasmuch as virus may be present in the wall of the bladder, it may be possible that virus may be voided with the urine.

The urine from ferrets, but not that of mink, was tested for virus. The data on the tests for virus in the urine of 7 infected ferrets included in table 2 show that DV was not demonstrable. Later tests of the ferrets, used for the virus demonstration attempts, revealed that they were susceptible to infection.

EXPERIMENT 2—FOMITES AND VECTORS

Pens.—Field observations and a few controlled laboratory observations indicate that pens and kennels that have housed diseased animals may remain infectious for a time after the infected occupant has been removed.[9] The quarters of veterinary hospitals and pet shops have frequently been incriminated as a source of infection for susceptible dogs.

In an attempt to show that contaminated pens may act as fomites, the following investigations are described:

Three small pens, designated A, B, and C, each of which had housed 2 moribund infected ferrets, were employed. The food and water dishes along with the food, water, and feces were left in place. Following the removal of the infected animals, 2 susceptible ferrets were placed in pen A immediately. Twenty-four hours later, 2 susceptible ferrets were placed in pen B and a week later 2 ferrets were placed in pen C. The test ferrets did not show signs of distemper during an observation period of 2 months.

A large pen approximately 18 by 3 ft. was used for housing an experimental epizoötic in which 20 ferrets became infected and succumbed. After the last ferret had succumbed and been removed, 4 susceptible ferrets were introduced. Although these ferrets remained in the pen for 50 days, no infection occurred.

Two months after the initiation of the test, all ferrets were found on inoculation to be susceptible to DV.

Mink-Handling Mitts.—All ranchers, fur graders, and judges use mitts or gloves to handle mink and ferrets. During handling, the animals often deposit saliva and nasal secretions on the mitts, and tests to determine whether they were contaminated with virus were initiated. This preliminary investigation indicated that DV survived for twenty minutes but not longer on contaminated gloves (table 3).

Flies.—Usually there is a large population of muscoid flies on every mink ranch during the warm months of the year. The isolation of distemper virus from flies has not been reported, yet the importance of arthropod vectors in the spread of other viruses suggests that flies may play a role in the dissemination of distemper.

TABLE 2—Inoculation of Ferrets with Urine Obtained from Infected Ferrets

Day after inoculation specimen collected	Dose per ferret (ml.)	Route of inoculation	Results*
12	3	i.p.	0/1
12	3	s.c.	0/1
15	2	s.c.	0/1
15	2	s.c.	0/1
18	2	i.p.	0/1
18	2	i.p.	0/1
18	2	s.c.	0/1

*Numerator indicates number of ferrets that died; denominator, the number inoculated.
i.p. = intraperitoneal; s.c. = subcutaneous.

In July, 1951, natural outbreaks of distemper occurred on two mink ranches in southeastern

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Wisconsin. Many mink on both ranches developed signs of distemper and muscoid flies were abundant. Screen flytraps were placed adjacent to pens of frank cases, and a large number of flies were collected within a short time.

TABLE 3—Subcutaneous Inoculation of Ferrets with Five Milliliter of Washings Collected from Contaminated Mink-Handling Mitts

No. and source of animals involved	Day of disease glove washings taken	Interval in minutes gloves held before virus recovery attempted	Results*
5 mink	17,19,21	1	1/2
5 mink	23,25,27	1	1/2
5 ferrets	21	10	1/1
5 ferrets	21	20	1/2
5 ferrets	21	30	0/2

*Numerator indicates number of ferrets that died; denominator, the number inoculated.

The flies were prepared for inoculation and 4 ferrets were injected subcutaneously. Two received 2 ml. of inoculum from one outbreak and 2 were injected with 2 ml. of inoculum from the other outbreak. Virus was not isolated from either pool.

Another attempt was made to isolate DV from muscoid flies during the summer of 1952. Flies were collected from a room that housed 12 infected mink. The flies were trapped on three alternate days, treated as previously described, and injected subcutaneously into 2 ferrets. No virus was demonstrated in this pool.

The 6 ferrets used in these trials were challenged later and found to be susceptible to distemper.

EXPERIMENT 3—METHOD OF TRANSMISSION

Contact.—Direct contact between infected and healthy animals may be the most effective means by which the virus is transmitted in nature. Aërosol transmission may be equally efficient if the distance traversed by the droplet projectiles is relatively short. Since transmission by means of contaminated mink-handling mitts has been accomplished, indirect contact must also be considered.

Research workers have experienced little difficulty in transmitting distemper infection by instilling the virus directly into the nostrils.[9] Distemper virus may be transmitted by artificially created aërosols with equally satisfactory results.[16, 19]

In order to ascertain objectively which mode of transmission may be the most efficient, two trials, A and B, were conducted. In trial A, the ferrets were confined to a single pen to provide an opportunity for transmission by direct and indirect contact and by the air-borne route. In trial B, the ferrets were placed in adjacent individual pens; presumably the spread of virus in this trial was only by aërial channels.

Trial A.—Twenty ferrets were placed in a single pen approximately 6 ft. long by 5 ft. wide which was situated in a room. One ferret, chosen at random, was inoculated subcutaneously with 1 ml. of a 10^{-1} dilution of DV, marked and returned to the pen. The small area forced the ferrets into intimate association, thus providing ready opportunity for spread of virus by direct and indirect contact. It is not possible to ascertain the relative importance of the several possible modes of transmission. The inoculated ferret died on the twenty-seventh day following injection. All of the remaining 19 contact ferrets became infected and eventually succumbed. The death times are recorded in figure 4.

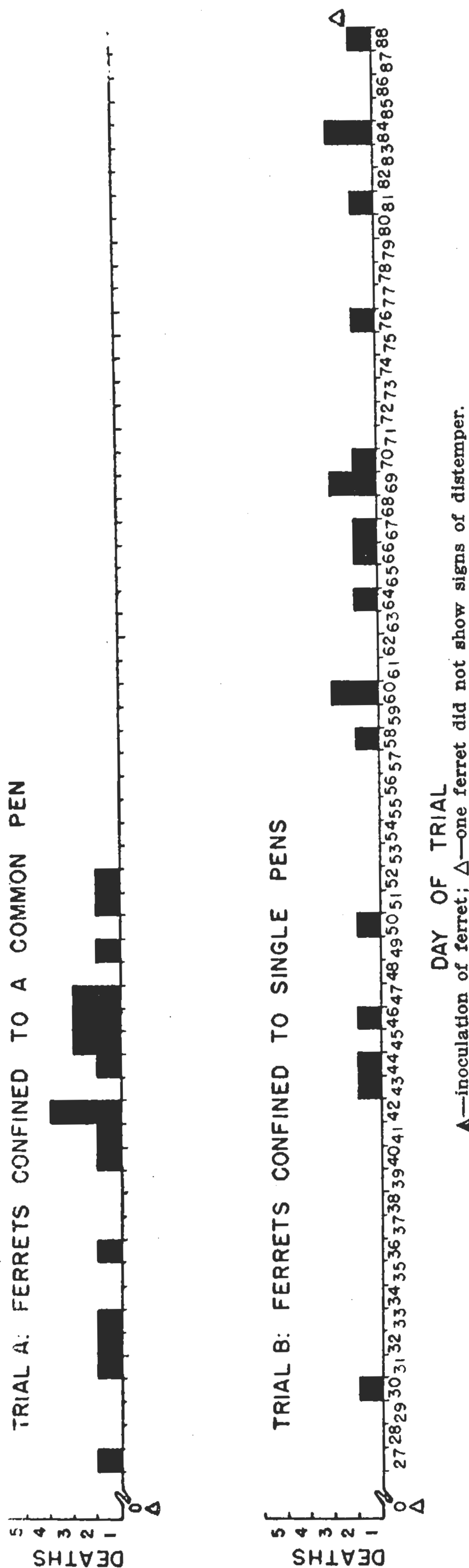
Trial B.—An attempt was made in this trial to compare the transmissability of DV by the air-borne route, when the possibility of direct and other indirect contact was eliminated. Each of 20 ferrets was confined to a single pen. These pens were arranged in rows of ten, one row above the other, but opportunity for direct contact among the animals was prevented. A ferret from one of the central pens was inoculated subcutaneously with 1 ml. of a 10^{-1} dilution of infective spleen tissue. It succumbed on the thirtieth day after injection. Subsequently, 18 ferrets died of the disease (fig. 4) and 1 failed to become infected. Through an oversight, this animal was not challenged.

In both trials, an auxilliary experiment revealed that the inoculated ferrets were shedding virus in their nasal secretions on the fifth day after subcutaneous exposure.

The distribution of deaths in the 2 trials indicates that the flow of the virus through the susceptible animals was much more rapid among those confined to a common pen than among those in individual pens. The combined data of two trials indicate that under the conditions of this experiment, spread by direct and indirect contact was more effective than by the aërial dissemination alone.

Aërial.—Preliminary trials were initiated to elucidate some of the factors concerned in the air-borne transmission of DV.

Trial A.—Efforts were made to demonstrate the dispersal and effective range of DV by droplet nuclei. As part of another experiment, a large outside woven wire pen (18 ft. by 3 ft.) housing 18 infected ferrets was used to provide the source of virus. On the area surrounding this pen, 4 small pens were placed at a yard's distance from each side of the large pen. In a similar arrangement, 4 pens were situated 10 yd., and 4 other pens 20 yd. from the pen containing the infected ferrets. Three pens were arranged at 35 yd. from 3 sides of the central pen. One ferret was placed in each of the small pens. None of the ferrets in this group showed signs of distemper within fifty days, whereas the ferrets in the central pen were all dead within twenty days after the trial was initiated.



▲—inoculation of ferret; △—one ferret did not show signs of distemper.

Fig. 4—Experimental epizoötics of distemper among ferrets.

Trial B.—In another experimental epizoötic in which individual ferrets were confined to single outside pens, the virus was spread from one infected individual to a healthy one through the air over a distance of 5 ft. Since the routine of feeding and of watering was in directly opposite order to the route of spread, it is believed that indirect transfer by caretaking procedures was precluded.

Trial C.—An artificial aërosol was created with a nebulizer. Three ferrets were exposed for one minute to a 10^{-1} dilution of infected ferret spleen. At the time of exposure, the gauge on the pressure line measured 3 lb. The 3 ferrets developed signs of distemper and were destroyed.

Trial D.—Because DV was detected in nasal exudate, an attempt was made to demonstrate virus in the expired air of infected mink and ferrets. Sinha *et al.*[27] described an air-sampling apparatus which permitted isolation of Newcastle disease virus proximate to infected birds, and this device was utilized in an effort to isolate DV from the air surrounding infected animals. In order to show that gelfoam did not have a detrimental effect on the virus, a lung suspension containing DV admixed with a disintegrated gelfoam pad. The titer of the mixture for ferrets was 10^5 while that of the suspension alone was 10^4.

Five ferrets were inoculated subcutaneously with 1 ml. of a 10^{-1} dilution of virus. Air was collected for five and ten minutes in approximately 25- and 50-liter quantities, respectively, during the third through the nineteenth day after exposure. The daily temperature of the room averaged 25 C. and the humidity, 71.2 per cent. Various samples were injected into 2 ferrets each: (1) a 50-liter sample collected from the room before the trial was started; (2) nine 25-liter samples collected on the fourth, fifth, sixth, eighth, tenth, twelfth, fifteenth, seventeenth and nineteenth days after exposure; (3) four 50-liter specimens obtained on the twelfth, fifteenth, seventeenth, and nineteenth days. None of the inoculated ferrets exhibited signs of DV infection, and each was found to be susceptible to subsequent challenge.

Trial E.—Air collected proximate to 12 infected young mink was processed as in the preceding trial. The temperature for the trial period averaged 24.7 C. and humidity 70.8 per cent. Thirteen 50-liter samples collected on the third, fourth, fifth, sixth, seventh, ninth, eleventh, fourteenth, seventeenth, twentieth, twenty-third, twenty-seventh, and forty-first day of the trial were each inoculated into 2 test ferrets, but none produced infection.

Trial F.—Failure to demonstrate DV in the gelfoam filter in trials D and E prompted lengthening of the collection time to sample larger volumes of air. An experimental epizoötic provided 15 infected ferrets. They were housed in a pen (6 ft. by 5 ft.) in a heated building. The collection funnel was placed in the center of the pen approximately 1 ft. above the heads of the

ferrets. One-hour, four-hour, and six-hour collections were made, which indicates that approximately 300, 1,200, and 1,800 liters of air, respectively, passed through the filter. The temperature and humidity were 29 C. and 27 per cent, respectively, at the time the samples were taken. Neither of 2 ferrets inoculated with each collection developed the disease.

Feeding.—The possibility that ferrets may be infected by feeding of DV-infected tissues was considered important because the malady might possibly be spread in nature by ingestion of virus-containing materials.

Trial A.—Before allowing ferrets to consume infected tissues, distemper virus was introduced directly into the stomach of ferrets. Each of 4 ferrets was administered 5 ml. of a 20 per cent spleen suspension with a stomach tube. The spleen had a titer of 10^{-4} m.l.d. per milliliter when inoculated subcutaneously in ferrets. The 4 exposed animals did not exhibit signs of distemper and all were found susceptible to later exposure.

Trial B.—Four ferrets were fed a minced infected liver-spleen mixture obtained from moribund ferrets. All of the material was consumed within six hours. Thirteen days later 1 of the ferrets showed signs of distemper, possibly through exposure of the respiratory tract during the process of feeding. The others remained normal and were found to be susceptible to challenge.

DISCUSSION

Study of the portals of elimination of infective DV by experimentally inoculated ferrets and mink demonstrated the virus in the nasal and conjunctival exudate and skin scurf. Virus was not obtained from the feces or urine. Detection of DV in the nasal exudate confirms the findings of others while demonstration in conjunctival exudate of infected animals may be the first.

The interest of the veterinary practitioner in clinical distemper begins with the onset of clinical signs. Distemper virus was demonstrated in the nasal exudate of mink and ferrets on the fifth day following inoculation but eye signs did not appear until the tenth or eleventh days. The virus persisted in the nasal exudate for eleven to fifteen days after the disappearance of eye, nose, and skin signs. That animals may be intermittent or transitory shedders of DV is a definite possibility. Repetition of this work and that with aërosol exposure may alter the particular time relationships which have been observed with subcutaneous injection. Most ferrets succumb to the malady while exhibiting catarrhal signs; therefore, the virus may be present in the nasal exudate until the time of death. Because of the possibility of carriers, control measures for distemper should consider the likelihood of a relatively long infectious period. Field outbreaks among mink point definitely to the occurrence of virus carriers.

Demonstration of virus in two of the three samples of skin scurf does not exclude the possibility that virus was shed with the scurf or was derived from saliva or other secretions. Obviously, other experiments must clarify this point.

Attempts to demonstrate virus in urine of infected animals were negative. Since distemper inclusion bodies are abundant in the bladder epithelium during the later stages of the disease and inasmuch as distemper virus has been demonstrated in the bladder tissue, it appears that there may be a possibility that the virus is masked or inactivated by urine.

Mesenteric lymph nodes may contain a high titer of virus.[24] Since distemper inclusion bodies are frequently present in the mucosa of the intestinal tract,[14] it is likely that the intestinal wall may contain virus. As with urine, the intestinal contents may inactivate or mask the virus and thus it is not demonstrable in the contents or feces.

The experiments designed to learn whether naturally contaminated pens may act as fomites were surprisingly negative. Since virus persisted on a mink-handling mitt for twenty minutes, it is likely that pens contaminated with infectious exudates and secretions may also carry the virus for that period. Chance undoubtedly played an important role in these trials. Mitts are seldom disinfected between handling of animals on mink ranches. Field observations of outbreaks indicate that handling of mink increases the spread of the malady.

Two pools of flies collected in the vicinity of infected mink did not contain virus. These trials are certainly not conclusive.

It is evident that the factors needed for the successful isolation of DV from the air were not fulfilled. Perhaps the air should have been sampled by passage through liquid in an impinger instead of through gelfoam.

Dunkin and Laidlaw[9] were the first investigators to provide evidence that the distemper virus was spread through the air for short distances in confined places. They believed that dilution by the external air prevented the transference of infection between pens. Significantly, Andrewes and Glover[31] (working with influenza virus) have suggested that increased ventilation,

or the interposition of a screen between their ferrets, appeared to have decreased the transfer of cross infection. They have shown that virus passed from 1 ferret to another across a distance of 5 ft. Distemper virus transversed a distance of 5 ft. in the present investigation.

Limited trials with alimentary tract infection were negative. The work of De Monbreun,[2] in which he indicated that puppies became infected by feeding on tissues containing virus, is open to question since he was probably dealing with infectious hepatitis virus. In the present study, the ferrets which were given the virus directly into the stomach by tube did not show signs of the disease.

In order to arrive at an accurate understanding of ecology of the distemper virus, serological tests must be employed in order to determine the prevalence of the disease in populations of carnivores. Interpretations made on clinical or pathological bases are not valid. Ott and co-workers[32] have shown that a large number of dogs, without a history of vaccination or disease, may have a high level of distemper-neutralizing antibodies in their serums. It is, therefore, probable that the distemper virus is perpetuated in nature by the carrier animal, although other possible vectors, or unrecognized hosts of the virus, can not be excluded at present.

SUMMARY

Results of experimental transmission trials with the Delavan strain of distemper virus among mink and ferrets were as follows:

1) Virus was first demonstrable in the nasal exudate of mink on the fifth day following inoculation and persisted from forty-six to fifty-one days.

2) Virus was detected in the nasal exudate of ferrets on the fifth day and persisted until the thirteenth day after inoculation.

3) The conjunctival exudate of ferrets was shown to contain virus on the sixteenth day following inoculation. Virus was present in conjunctival exudate collected from mink on the twenty-first and thirtieth day post-inoculation.

4) The skin scurf of infected mink and ferrets obtained late in the course of the disease contained distemper virus in infectious quantities.

5) Virus could not be demonstrated in the feces of infected mink or the urine or colon contents of infected ferrets.

6) Virus was not demonstrable in pens that had housed infected ferrets.

7) The virus persisted on handling mitts for twenty minutes but not thirty minutes.

8) The virus could not be detected in flies trapped near infected mink.

9) An attempt was made to compare the efficacy of natural transmission mechanisms.

10) Ferrets were infected with an artificially produced aërosol containing distemper virus (DV).

11) An unsuccessful attempt to demonstrate DV from the air proximate to infected mink and ferrets was described.

12) Ferrets did not become infected following the administration of virus directly into their stomachs. One of 4 ferrets became infected in a feeding experiment.

13) The transmission and epizoötiology of DV was discussed.

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