PODODERMATITIS IN FARMED MINK IN CANADA

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ABSTRACT

PODODERMATITIS IN FARMED MINK IN CANADA

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An apparently new skin disease affecting the feet of farmed mink (*Mustela vison*) was observed in eastern and central Canada in the spring of 1996. Epidemiologic studies showed that the disease prevalence varied from 0.8% to 69% among farms, that males were preferentially affected and that there was an association between the condition and the feeding of harp seal meat (*Phoca groenlandica*). The lesions ranged from alopecia, swollen pads, mild hyperkeratosis and thick skin folds around toes; to ulcers, crusts and marked hyperkeratosis. Microscopically there was hyperkeratosis and a wide spectrum of follicular inflammation. Bacterial cultures were mixed but *Staphylococcus intermedius* predominated. Virus isolation attempts were negative as was immunohistochemistry for morbillivirus. One out of 42 analyzed mink sera contained antibodies against calicivirus (San Miguel sea lion virus 5, 13, 17). The study suggests that the cause of pododermatitis in mink is an, as yet unidentified, infectious agent with secondary bacteriologic pyoderma.

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we will love only what we understand,
we will understand only what we are taught"
- Baba Dioum, Senegal

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DECLARATION OF WORK PERFORMED

I declare that, with the exception of the items indicated below, all work reported in this thesis was performed by me.

Sampling of mink was undertaken with the assistance of Dr. D. B. Hunter and Dr. E. K. Martin. Preparation of histology slides was performed by the histology laboratory, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada. Dr. Philip Byrne helped in the preparation of tissue for electron microscopy and Dr. S. Yamashiro helped looking at the tissues under the electron microscope. Distemper immunohistochemistry was performed by Dr. D. Haines, Immunohistochemical Services, Diagnostic Immunology Laboratory, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Canada. Calicivirus serology was performed by Dr. A. Smith, Calicivirus Laboratory, College of Veterinary Medicine, Oregon State Medicine, Oregon State University, U.S.A. Aleutian Disease serology was performed by the Animal Health Laboratory (AHL), University of Guelph, Guelph, Ontario, Canada. Virus isolation attempts were performed by Dr. A. Smith and by the AHL. Assistance with analysis of the data was provided by Dr. D. B. Hunter, Dr. I. K. Barker, Dr. J. Prescott and Dr. M. Thorburn.

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PODODERMATITIS IN FARMED MINK IN CANADA

CHAPTER 1

1.0 General Introduction

In the spring of 1996, mink farmers in central and eastern Canada first reported what they believed was a "new" disease affecting the feet of their mink (*Mustela vison*). The lesions consisted of ulceration and hyperkeratosis of the footpads and skin on the plantar and volar aspects of the metatarsal and metacarpal regions. In many mink, the junctional areas between footpads and furred skin were most affected, while in other animals the nail beds and toes were ulcerated. Occasional mink had lesions on the nose pads, corners of the eyes and on the head. The major economic concern for mink farmers was that affected male mink failed to breed, apparently due to the painful ulcers on the feet. Some of the mink recovered after antibiotic treatment, while others recovered without treatment. Many of those that recovered relapsed a few months later. Mink farmers associated the disease with the feeding of seal meat as part of the mink ration.

Mink are carnivores and require a high protein diet (Atkinson, 1996). Maintenance rations should provide 30% of the metabolizable energy in the form of protein, while 35% protein is required during gestation and 40% protein during lactation (Atkinson, 1996). Protein is the most expensive component of the mink ration and farmers are constantly trying to find less expensive, alternative protein sources. Seal meat, a by-product of the east coast Canadian sealing industry, is a good source of relatively inexpensive, but high quality, protein. Mink farmers in eastern Canada began using seal

meat in the mink ration in the late 1990's, which coincided with the first reports of pododermatitis in commercial mink.

There is historical evidence that seal meat can cause infection and disease in mink. Commercial mink raised in Utah, USA in the early 1970's, developed similar foot pad lesions after being fed meat from Northern fur seals (*Callorhinus ursinus*) harvested from the west coast of Alaska, USA (Larsen, 1997). No reports were published on this condition, and all historical records and photographs were destroyed when the Utah Fur Cooperative offices were bombed by animal rights activists.

The possibility that seal meat was the cause of the pododermatitis on the Canadian mink farms prompted the Canada Mink Breeders' Association to approach the Ontario Veterinary College for help in investigating this disease. The research presented in this thesis provides information on the epidemiology and pathology of the disease. Although several possible etiologic agents have been ruled out, the cause of the disease is still undetermined and further research is warranted.

1.1 Statement of Goals and Hypotheses

The goals of the study were to describe the epidemiology and pathology of this new disease and determine if there is an association with the feeding of east coast seal meat. The specific working hypotheses were as follows:

1. There is an association between pododermatitis in mink and the feeding of seal meat.
2. The pathogenesis of the disease involves an infectious agent.

The three main objectives of the research were to:

1. describe the epidemiology of the disease and determine any association with the feeding of seal meat. A retrospective "mail-out" survey and a prospective "on-farm"

monitoring program were used to determine the prevalence of the disease at pelting time, when large numbers of animals were available for inspection.

2. describe the macroscopic and microscopic lesions found in affected mink including live sick animals, mink that died or were euthanized due to the condition and mink examined at pelting time.

3. identify the possible etiology of the disease. Histology (including special stains), immunohistochemisty, electron microscopy, bacteriology, serology and virology were used as tools to search for a possible etiologic agent.

1.2 Literature Review

The pododermatitis observed in farmed mink in Canada from 1996 is an apparently new disease. There are no published records describing foot pad lesions similar to those reported in mink. There is unpublished historical evidence (Larsen, 1997) indicating that a similar disease outbreak occurred in farmed mink in Utah, USA in the early 1970's, which was thought to be associated with feeding meat from Northern fur seals.

This literature review focuses on diseases reported to cause lesions of the feet of mink, and on diseases of the feet of other animal species that present similarly to the mink disease. In addition, as seal meat added to the mink ration is the putative cause of the disease, infectious agents that could be present in seal meat and cause similar lesions are also reviewed.

1.2.1 Diseases of mink that affect footpads

Distemper

Canine distemper virus (CDV) is a morbillivirus within the *Paramyxoviridae* family. Though it is not specifically epitheliotropic, the virus causes cutaneous lesions (Crook et al., 1958). The disease in farmed mink is controlled by vaccination. However, it is important to note that vaccines derived from avian cell culture can cause disease in mink (Sutherland-Smith et al., 1997).

The virus has a global distribution and infects a wide range of hosts (Barrett, 1999) including North American mammals such as raccoons (*Procyon lotor*) and striped skunks (*Mephitis mephitis*), which are common visitors to mink farms. Outbreaks of distemper occur every year in the mink industry despite prophylactic vaccination.

Transmission is by direct or indirect contact or by aerosol over short distances (Budd et al., 1966). The incubation time is usually nine to 14 days in aerosol-infected mink (Crook et al., 1958) but may be longer. The virus replicates in the respiratory system, invades the blood stream soon after, and causes a multi-systemic disease. Clinical signs begin with watery eyes and serous discharge from the nose. The discharges then become purulent and can completely cover the eyes and nose (Pearson and Gorham, 1987). Anorexia, depression, and hyperemia of the skin around the foot pads and muzzle ensues. As the disease progresses, the skin lesions become thickened and hyperkeratotic. The disease is often referred to as "hardpad disease" because of the characteristic hyperkeratotic foot pads (Hunter, 1996). Some mink can die within a few days after the onset of clinical disease. Others may improve but after ten days to five weeks many develop an encephalitis which leads to episodes of vocalization ("screaming fits"), convulsions, and death within two days (Pearson and Gorham, 1987).

Gross lesions in mink dead of distemper are limited. The skin may be hyperemic and foot pads and nose may be hyperkeratotic. Many animals have enlarged spleens and the lungs are often congested or mottled red and tan (Pinkerton, 1940 in Pearson and Gorham, 1987). Histologic lesions are more common and are characteristic. In the lung there is inflammation which ranges from acute, multi-focal interstitial pneumonia with bronchiolitis to diffuse interstitial pneumonia. Occasional syncytial cells are also seen. Early in the disease, eosinophilic intracytoplasmic (and occasionally intranuclear) inclusion bodies are seen within epithelial cells of the lung (Crook and McNutt, 1959). Lesions in other organs include lymphoid depletion, hyperkeratosis of the skin, and inclusion bodies in a variety of tissues. Mink with neurologic signs have a

nonsuppurative encephalitis. The histologic picture can be complicated by secondary bacterial infections, especially in the lungs and skin.

Bacterial infections

Bacterial infections of the skin are relatively common in individual mink but seldom cause a herd problem (Onderka, 1996). The bacteria most often associated with skin infections in mink are *Staphylococcus* spp. and *Streptococcus* spp. *Staphylococcus aureus* has, for example, has been isolated from young mink kits with dermatitis on the head, neck, and perineum (Crandell et al., 1971) and *Staphylococcus intermedius* was isolated from neonatal kits with dermal adenitis (Hunter and Prescott, 1991). Similarly, in dogs *Staphylococcus intermedius* and *Staphylococcus aureus* are the most commonly isolated bacteria from healthy and infected skin (White et al., 1983; Berg et al., 1984; Cox et al., 1984). Other bacteria such as *Proteus* spp, *Pseudomonas* spp and *E. coli* are usually secondary invaders in infections initiated by coagulase-positive staphylococci (Ihrke, 1987).

Factors that predispose to pyoderma include seborrhea, allergy, poor grooming, ectoparasites, immune incompetence and endocrinopathies (Ihrke, 1987). Follicular hyperkeratosis, common to many chronic skin conditions, predisposes to bacterial colonization of hair follicles and folliculitis (Yager and Scott, 1993). Ihrke (1987) classifies canine pyodermas based on the depth of involvement of the skin (surface, superficial and deep pyodermas). Surface pyodermas include acute moist dermatitis and skin fold pyodermas. Superficial pyodermas are the most common bacterial skin diseases. The clinical presentation includes pustules, crusted papules, erythematous plaques, hyperpigmentation, and alopecia. Deep pyodermas are less common than superficial pyodermas. Dogs can present with nodular pustules, hemorrhagic bullae,

fistulae, seropurulent debris, hyperpigmentation, swelling and ulcers. Underlying disease is common in deep pyodermas.

Hereditary tyrosinemia

Hereditary tyrosinemia (HT) was originally called "pseudodistemper" since certain aspects of the clinical disease, such as ulceration of foot pads and nose, resemble canine distemper (Schwartz and Shackelford, 1973). HT is an inherited, autosomal recessive disease affecting only dark color phase mink. Affected mink have elevated serum tyrosine levels due to a deficiency of the liver enzymes that normally transaminate tyrosine, tyrosine aminotransferase or 4-hydroxyphenylpyruvate dioxygenase (Goldsmith, 1978). The lesions result from an inflammatory response to tyrosine crystals deposited in tissues (Goldsmith, 1978).

The first signs are watery eyes and diffuse corneal clouding, conjunctivitis, corneal ulceration and necrotizing dermatitis of the foot and nose pads (Christensen et al., 1979). In the spring form of the disease, affecting very young animals, the mink stop eating, waste, and die within 2-3 days after onset of clinical signs. The fall form involves mink which do not develop symptoms until they are around six months of age. The signs are less severe and the mink survive several months (Christensen et al., 1986).

The histological and macroscopic lesions of HT have been described by Goldsmith (1978). On post-mortem examination the ulcerated feet and inflamed eyes are evident. Kidneys are often pale, decreased in size, and have an irregular capsular surface. The footpad lesions are characterized by folliculitis, basal cell necrosis, and inflammation often associated with granules or crystals of tyrosine (Muller et al., 1989 d). There may be corneal edema and sometimes ulceration with chronic suppurative stromal keratitis (Goldsmith, 1978). Kidneys have a chronic interstitial nephritis with dilated tubules due

to fibrosis and back up of urine. Focal mineralization and renal papillary necrosis may also be present.

Zinc deficiency

Zinc is an essential trace element present in all body tissues, especially muscle, bone, teeth and hair. Zinc is a component and cofactor of many enzymes and plays a role in muscle and bone growth, the metabolism of protein and carbohydrates, keratogenesis and wound healing (Kunkle, 1980; Sanecki et al., 1982).

In dogs there are two syndromes of zinc-responsive dermatosis (Kunkle, 1980; Fadok, 1982; Ohlén, 1986; Muller et al., 1989 e). The first syndrome is mostly seen in certain arctic breeds and appears to be an inherited condition and, therefore, does not apply to mink. The second syndrome occurs in growing puppies and is associated either with a zinc-deficient diet or with a relative zinc-deficiency associated with diets high in cereals (phytates) or minerals (especially calcium) that interfere with the uptake of zinc. The clinical appearance varies, and includes hyperkeratosis, scales, crusts, erythema and exudation. Lesions are seen on pressure points, distal extremities, footpads, planum nasale and trunk (Kunkle, 1980; Ohlén, 1986; van den Broek, 1986). Secondary pyoderma with primarily *Staphylococcus* sp. and an associated lymphadenopathy is occasionally observed (Ohlén, 1986; Van den Broek, 1986). Microscopic changes include acanthosis, diffuse and follicular parakeratotic hyperkeratosis, vacuolation of basal cells, pigmentary incontinence, degeneration of follicles and mild neutrophil, mononuclear or eosinophil-rich infiltrates (Sanecki, 1982; Van den Broek, 1986; Wolf, 1987). In cases with secondary bacterial infection, varying degrees of pustular dermatitis and folliculitis are present (Yager and Scott, 1993).

Zinc deficiency is mentioned by Onderka (1996) as a possible cause of skin and foot pad lesions of mink. However, according to Atkinson (1996), trace elements such as zinc, copper, iodine, and selenium are generally present in adequate amounts in conventional mink diets. This, of course, assumes that the ration is properly formulated so that the absorption of zinc is not impaired.

1.2.2 Pododermatitis in other species

In a review of pododermatitis in small animals, White (1989) divides the potential causes of pododermatitis into infectious, allergic, autoimmune, endocrine, environmental and psychogenic. An additional group consists of hyperkeratotic, nodular and pigmentary dermatoses.

Infectious disease

Infectious pododermatitis in dogs and cats can be caused by bacteria, dermatophytes, intermediate mycoses, parasites and viruses. Bacterial infections of the feet often are secondary, and are dominated by *Staphylococcus intermedius* infection as described in the section on dermatoses affecting the feet of mink.

Dermatophytes can affect the feet of dogs and cats (White, 1989). Dermatophyte infection occasionally has been reported in mink (Finley and Long, 1978). The dermatophytes that most commonly affect animals are *Microsporum sp.* and *Trichophyton sp.* (Holfeld et al., 1980). Dermatophytes spread by direct or indirect contact with infected animals or objects (Medleau and White-Weithers, 1991). Factors that predispose cats to infection include compromised immune status (which makes kittens more susceptible than adults), debilitating disease, poor nutrition and stress (De Boer and Moriello, 1995).

Dermatophytes live on keratin and therefore are found in the dead, cornified layers of the skin including hairs, nails, and stratum corneum (De Boer and Moriello, 1995). Dermatophyte spores colonize the stratum corneum and develop into hyphae. The hyphae invade hair follicles and, with the help of keratinases, produce spores either within the hair (endothrix type) or along the outer surface of the hair (ectothrix type) (Holfeld et al., 1980; Medleau and White-Weithers, 1991). The infection leads to hyperplasia of the epidermis with production of rete ridges as well as ortho- and parakeratotic hyperkeratosis (Yager and Scott, 1993). The hyperkeratosis predisposes to secondary bacterial infection leading to folliculitis or furunculosis. The clinical signs are variable including alopecia, erythema, scaling, inflammatory plaques, and nail abnormalities (White, 1989; De Boer and Moriello, 1995). Occasionally the only lesion observed is a pyogranulomatous inflammation of the dermis caused by a hypersensitivity reaction to the dermatophytes (Holfeld et al., 1980). The histologic picture is as variable as the gross lesions. Muller et al. (1989 b) divide the histologic lesions into three major patterns: 1) perifolliculitis, folliculitis, and furunculosis; 2) perivascular dermatitis with orthokeratotic or parakeratotic hyperkeratosis of the epidermis and hair follicles; 3) intraepidermal vesicular or pustular dermatitis. Fungal hyphae, as well as spherical and oval conidia, may be present within the stratum corneum, the hair follicles, or around the hairs.

Intermediate mycoses (mycetoma and sporotrichosis) and systemic mycoses (blastomycosis) sometimes can cause pedal dermatoses (White, 1989). Mycetomas are rare, chronic, granulomatous lesions that lead to subcutaneous swellings (Brodey et al., 1967). They are often associated with infection of a traumatic lesion (Muller, 1989 b). The etiologic agents involved are filamentous bacteria (actinomycotic mycetoma) and

fungi (eumycotic mycetoma) (Yager and Scott, 1993). In dogs the lesions occur most often on the feet. They begin as tender, painful, swollen areas. Draining fistulas that discharge serosanguinous or purulent fluid subsequently develop. On cut surface there is dense gray-white connective tissue with necrotic areas containing fungal granules (Yager and Scott, 1993; Muller, 1989 b) . Each species of fungus, with few exceptions, has its own distinctive type of granule with variations in size, color, shape, and architecture (Brodey et al., 1967). Histologically there is diffuse or nodular dermatitis. There are fungal granules and occasionally epithelioid macrophages amongst fibrotic, chronically inflamed connective tissue stroma (Yager and Scott, 1993).

Sporotrichosis is an uncommon chronic, granulomatous infection caused by the dimorphic fungus *Sporothrix schenckii* (Scott et al., 1974). The organism usually enters through wounds in the skin and spreads via the lymphatics. Infection results in subcutaneous nodules or granulomas that ulcerate and discharge a thick, brownish-red exudate (Scott et al., 1974; Carter, 1986) Histologically, there is a diffuse and/or nodular pyogranulomatous dermatitis and panniculitis (Koehne et al., 1971; Yager and Scott, 1993). There are microabscesses, macrophages, multinucleated giant cells, lymphocytes and plasma cells around the fungi. The fungi are, however, often difficult to find, even with special stains.

Blastomycosis is a systemic disease caused by the dimorphic fungus *Blastomyces dermatitidis*. (Legendre et al., 1981). Clinical signs associated with blastomycosis reflect the multisystemic nature of the disease (Arceneaux et al., 1998). The body systems commonly involved are respiratory, lymphatic, cutaneous and ocular. In two retrospective studies of blastomycosis in dogs, 26% of 47 cases and 52% of 115 cases had skin involvement (Legendre et al., 1981; Arceneaux et al., 1998). Skin lesions are

ulcerative or granulomatous, sometimes with draining tracts (Legendre et al., 1981; White, 1989). Since the disease is multisystemic, it is an unlikely cause of the mink pododermatitis.

Parasites that can lead to lesions on the feet include certain hookworm larvae (e.g. *Ancylostoma sp.*), saprophytic nematode larvae (e.g. *Pelodera strongyloides)* and mites (e.g. *Demodex canis*) (White, 1989). Since both hookworms and *Pelodera sp.* live in damp soil, inaccessible to farmed mink, these conditions will not be discussed further.

Demodectic mites can be found in small numbers in the hair follicles of most animal species (Gafaar, 1958; Nutting and Desch, 1978). In certain individuals, mites proliferate and lesions of demodectic mange develop. This proliferation is probably related to compromise of the dogs' immune system (Caswell et al., 1995). Canine demodicosis can be localized or generalized (Lemarie, 1996). The localized form consists of small circumscribed, erythematous, scaly, non-puritic areas of alopecia most commonly on the face and forelegs (Muller, 1989 a). Generalized demodicosis is similar to the localized form but affects large areas of the body (Lemarie, 1996; Lemarie et al., 1996). Generalized demodicosis can become pustular when secondary pyoderma is present and there may be concurrent foot lesions with hyperkeratosis. The histological picture of demodicosis varies with the degree of secondary bacterial infection but is mainly characterized by a lymphocytic-plasmacytic mural folliculitis, nodular dermatitis, and suppurative furunculosis (Caswell et al., 1995). Hair follicles are often hyperkeratotic and contain variable numbers of mites.

Except for canine distemper virus infection, virus-induced dermatoses are rare in cats and dogs (Muller et al., 1989 a). Poxvirus (Bennett, 1985 and Gaskell et al., 1983), feline herpesvirus (Flecknell et al., 1979) and feline calicivirus (Cooper and Sabine, 1972) have

been reported to cause dermatoses involving the feet of cats. The feline poxvirus is an orthopoxvirus related to cowpox virus (Gaskell et al., 1983). Clinical signs include ulcerated, nodular lesions often on the paws (periungual areas or footpads) and sometimes on the face, limbs and base of the tail (Gauguerre et al., 1992). Secondary cutaneous lesions are multiple nodules that develop into papules or plaques and ulcerate. The ulcers heal over a two to three week period. Secondary bacterial infection may occur. The histologic lesions are focal, sharply demarcated ulcers covered by a fibrinonecrotic inflammatory exudate that may extend into the deep dermis (Yager and Scott, 1993). Eosinophilic intracytoplasmic inclusion bodies occur in keratinocytes, in epithelium at the edge of ulcers and in the epithelium of the external root sheath.

Feline herpesvirus infection usually is associated with upper respiratory tract disease. However, a feline herpesvirus has also been associated with ulcerative foot pad lesions (Flecknell et al., 1979). Observed lesions were multiple and varied in diameter between 1 and 2 cm. Early lesions were red, ulcerated and moist, while older lesions were dry with thick scabs. Skin biopsies revealed intraepidermal microabscesses and focal necrosis of the epithelium of hair follicles, as well as an inflammatory reaction in the dermis. No intranuclear inclusion bodies were seen. Bacterial culture of the lesions yielded growth of "*Staphylococcus pyogenes*" in two cases and "*Streptococcus epidermidis*" in one case. Herpesvirus was isolated from skin and oral swabs.

Feline calicivirus has been reported as the cause of "paw and mouth disease" in a cat (Cooper and Sabine, 1972). Macroscopic lesions included erosions of the pads of both right feet and inflammation of the volar and plantar interdigital areas as well as blisters on the tongue, palate, and lips. No virus particles were observed on direct examination of blister crusts with an electron microscope. However, feline caliciviruses were isolated

both from the tongue and paw lesions. It was suggested that the paw lesions may have spread from the mouth during self-grooming (Cooper and Sabine, 1972).

Papillomatous digital dermatitis of cattle is an apparently contagious, painful, circumscribed dermatitis of the feet of cattle (Walker et al., 1995). Lesions are most often seen on the volar aspect of the hind feet in the interdigital space and on the heel bulb. Early lesions are circular to oval (0.5 to 1 cm in diameter), alopecic, red, flat and ulcerative. Older lesions are larger (2-4 cm in diameter) with raised, wart-like papillary projections (Read et al., 1992; Walker et al., 1995). The condition has primarily been associated with spirochetes. Borgmann et al. (1996) described the microscopic lesions as hyperkeratosis, acanthosis, micro-abscessation and neutrophilic inflammation of the epidermis. The superficial dermis was congested and contained a mixed inflammatory reaction. The deep dermis was congested and there was perivascular and periadnexal infiltration with lymphocytes, plasma cells and occasional eosinophils and neutrophils. Spirochetes were mainly seen in the epidermis.

Allergic, endocrine and autoimmune diseases

Allergic, endocrine and autoimmune diseases can lead to pododermatitis in dogs and cats, but are on epidemiological grounds unlikely causes of the pododermatitis studied in Canadian mink between 1997 and 1999 and will therefore not be discussed in detail.

Allergic contact dermatitis is an uncommon cause of pedal dermatoses in dogs and cats (White, 1989). It is a cell-mediated, type IV hypersensitivity reaction with a long (six months to two years) sensitization period (Kunkle, 1988). Lesions consist of erythema, papules, exudative or ulcerated plaques, alopecia and pruritus on the underside of the feet (White, 1989; Gauguerre et al., 1992). Since pododermatitis in the mink in the

present study was seen in many young animals, allergic hypersensitivity is not a probable cause.

Endocrine diseases that can lead to foot lesions include hypothyroidism and hyperglucocorticoidism. The endocrinopathies can predispose to bacterial or dermatophyte infections (White, 1989), but there are usually also signs of systemic disease (Scott, 1982; Spearman and Little, 1978). Endocrine diseases are unlikely to affect many animals on several farms at the same time unless medication or a component in the feed interferes with the normal metabolism of the endocrine systems.

In dogs and cats autoimmune cutaneous diseases such as pemphigus foliaceus can present with footpad lesions as the only clinical signs (August and Chickering, 1985; Ihrke et al., 1985). Grosslesions include marked hyperkeratosis and villous hypertrophy of the footpads, occasionally with peeling, fissuring, swelling, and ulcerations. The microscopic lesions were characterized by hyperkeratosis, subcorneal acantholysis, degenerating inflammatory cells in crusts, and perivascular, mixed inflammatory cell infiltrates in the superficial dermis.

Environmental disease

Environmental diseases affecting the feet include irritant contact dermatitis, thallium toxicity and lesions caused by trauma and friction (White, 1989). Irritant contact dermatitis occurs when an irritant or toxic substance is applied to the skin. Substances that can result in irritant contact dermatitis include soaps, detergents, disinfectants, insecticides, solvents, fertilizers, diesel oil and strong corrosive acids or alkalis (Kunkle, 1988). Most skin irritants cause erythema, papules and occasional vesicles. Microscopically there is spongiosis of the epidermis with a lymphocytic infiltrate. In chronic cases the cellular infiltrate is mixed (Kunkle, 1988).

Thallium, a heavy metal used as a rodenticide, can produce lesions in a number of body systems including the skin. Clinical signs often start with vomiting, anorexia and diarrhea (Lennox, 1966; Withers, 1972; Ruhr and Andries, 1985). Cutaneous lesions can be the only presenting sign when the exposure to thallium is of long duration (Zook and Gilmore, 1967). The lesions in the skin often begin at the commisures of the lips or nasal cleft and extend over the chin, face and inner pinnae of the ears. Lesions also develop on the footpads, toes, limbs, neck, axillae, flanks, ventral abdomen and perineum (Lennox, 1966; Zook et al., 1968; Withers, 1972; Ruhr and Andries, 1985). Affected skin is first erythematous, then serum begins oozing from the lesions, scaling and crusting develops, and hair starts falling out. In some cases, the thickened foot pads resemble "hard pad" disease (canine distemper) (Zook and Gilmore, 1967). Microscopically there is parakeratotic hyperkeratosis, spongiosis, intraepithelial abscessation and necrosis of the epidermis (Lennox, 1966; Zook and Gilmore, 1967; Zook et al., 1968). The lesions in the dermis consist of congestion, edema and infiltration of neutrophils and mononuclear cells. Thallium toxicity usually has a poor prognosis.

Traumatic skin lesions may result in secondary pyodermas and/or introduction of keratin into the dermis, where the keratin will function as a foreign body (White, 1989). Friction or pressure in a localized area of the skin, especially over bony prominences, can lead to the development of callosities (Yager and Scott, 1993). The skin responds to the pressure with epidermal proliferation with prominent hyperkeratosis. Macroscopically the callosities are well-circumscribed, raised, alopecic, gray, keratinous plaques. The callosities can be secondarily infected with bacteria.

An example of pododermatitis due to friction and traumatic injury is seen in rabbits. Ulcerative pododermatitis in rabbits, often referred to as "sore hocks", is a traumatic

lesion of the plantar surface of the metatarsal region and less commonly the volar surface of the metacarpal-phalangeal region (Flatt, et al., 1974). The lesions consist of circumscribed skin ulcers covered by red-black, dry crusts (Marcato and Rosmini, 1986). Predisposing factors include heavy body weights, excessive nervousness in rabbits causing them to stamp their feet frequently, the use of wire-floored cages and stagnation of urine in the bedding (Flatt, et al., 1974; Marcato and Rosmini, 1986). The lesions begin with hyperplasia of the epidermis and result in ulcers due to ischemic necrosis of the skin. Often the ulcers are secondarily infected with bacteria, especially *Staphylococcus aureus* or *Fusobacterium necrophorum*, resulting in abscesses under the crusts. A similar condition occurs in guinea pigs (Sirois, 1989).

Hyperkeratotic, nodular and pigmentary disease

Hyperkeratotic, nodular and pigmentary diseases include zinc-responsive dermatoses, digital hyperkeratosis, sterile pyogranulomas, plasma cell pododermatitis, vitiligo and neoplasia. Zinc-responsive dermatitis was discussed above.

Digital hyperkeratosis occurs spontaneously in some adult dogs (Muller, 1989 e). It preferentially affects the periphery of the pads where haired and non-haired parts join. Often the pads have fissures and erosions which makes walking painful. The cause is unknown.

Sterile pyogranulomas are sometimes seen in dogs and less commonly in cats (Muller, 1989 c). The animals have firm, non-painful, non-pruritic dermal papules, plaques and nodules mainly on the face and/or feet (Yager and Scott, 1993). The lesions may become alopecic, ulcerated, and secondarily infected. Histologically there is granulomatous to pyogranulomatous dermatitis along the hair follicles but the follicles are not invaded. There are no microbial agents and no foreign material can be identified.

Plasma cell pododermatitis is a rare disease of cats (Taylor and Schmeitzel, 1990). The lesion begins as a soft, non-painful swelling of the foot pad, which may ulcerate (Gruffydd-Jones et al., 1980). The microscopic lesions include perivascular diffuse dermatitis with plasma cells predominating (Taylor and Schmeitzel, 1990).

Vitiligo is an idiopathic loss of pigment which can affect the footpads. It is a benign disease without systemic signs (White, 1989).

Neoplasia is not a probable cause of disease in large numbers of animals during a limited time period (unless induced by an infectious or environmental agent).

1.2.3 Possible infectious agents in seal meat that could result in mink pododermatitis

Many of the mink with pododermatitis had been fed seal meat from the east coast of Canada. The major seal species hunted in this area is the harp seal (*Phoca groenlandica*). Since mink farmers believed there could be an association between the mink pododermatitis and feeding seal meat, transmissible, infectious agents that could be present in seals are of interest. Among these agents are phocine distemper, calicivirus, poxvirus, and herpes virus.

Phocine distemper virus (PDV) is a morbillivirus that was discovered in conjunction with an epizootic that killed large numbers of harbor seals (*Phoca vitulina*) in western Europe in 1988 (Kennedy, 1990). It is probable that PDV was derived from CDV (Barrett, 1999). Clinical signs are similar to those of canine distemper and include respiratory, gastrointestinal, and neurological signs. On post mortem examination the major finding is pneumonia. Microscopic lesions include bronchopneumonia, cytoplasmic eosinophilic inclusion bodies in bronchial epithelium, intra-alveolar macrophages, and multinucleated syncytia. In the brain there often is a non-suppurative

demyelinating encephalitis, and intracytoplasmic inclusion bodies can be seen in neurons (Kennedy et al., 1989; Kennedy, 1990).

After the European epizootic, PDV and antibodies against PDV have been found in numerous marine mammals including seals on the eastern coast of Canada (Duignan, et al., 1997). Harp seals appear to be relatively resistant to clinical distemper, however, morbillivirus encephalitis has been documented from a juvenile harp seal in the Gulf of St. Lawrence, Canada (Daoust et al., 1993).

Mink have been experimentally infected with morbillivirus derived from diseased harbour seals (Blixenkrone-Møller et al., 1989). The infection resulted in an acute disease resembling the acute systemic and nervous form of canine distemper. Furthermore, some authors suggest that the virus causing the epizootic in seal in the North Sea in 1988 may have infected mink on land, or alternatively, that the virus in the sea may have originated from virus-infected mink (Örvell et al., 1990). It is thus possible that distemper virus can be transmitted to mink fed meat from infected seals.

Caliciviruses of marine origin (San Miguel Sea Lion Virus, SMSV) were first isolated in 1972 from California sea lions (Smith and Boyt, 1990). They have since been isolated from several marine mammals off the western coast of North America. Infection with SMSV produces vesicular lesions on the flippers of marine mammals. Experimental infection of swine with SMSV produces vesicular lesions indistinguishable from those of vesicular exanthema (Smith and Boyt, 1990). Blisters are most prominent on the dorsal surfaces of the fore flippers and contain either clear fluid or purulent material (Smith, 1987 b). The blisters range in diameter from 1mm to 3cm and usually erode, leaving shallow, rapidly-healing ulcers. Virus can be isolated from throat and rectal swabs. Experimental infection of harp seals with SMSV type 2 indicated that they were

susceptible to infection, but the resultant disease was inapparent or mild and self-limiting (Gelberg et al., 1982).

In 1974 Sawyer et al. (1978) observed that 2% of northern fur seals harvested around St. Paul Island, Alaska had vesicular lesions on their flippers. The seals were being harvested for pelts and subsequently processed for use as mink feed. Eight seal carcasses with vesicular lesions were processed in the same manner as carcasses destined for mink feed. The head, stomach and intestines were removed and then the remaining portion of the carcass, including organs and flippers, was ground up and examined for the presence of viruses. San Miguel seal lion virus (SMSV) was isolated from four of the eight carcasses. Since potentially infected seal meat was being used as mink feed, mink were experimentally fed seal meat infected with San Miguel sea lion virus and were injected intradermally with the same virus to study its effect on mink (Wilder and Dardiri, 1978). The mink fed the infected meat did not show any clinical signs but virus was isolated from the rectum of one mink and two mink showed a twofold increase in serum titer. One of the mink that had been inoculated intradermally had a febrile response and a ruptured vesicle at the inoculation site.

Caliciviruses are not heat stable and can be killed by NaOH (Smith, 1987 b). However, they seem to tolerate quick freezing (Sawyer et al, 1978) and feline picornavirus (presently known as calicivirus) is reportedly relatively stable and will survive up to eight days in a dry environment and ten days in a damp environment (Love, 1972). Taking into account the information above, it is possible that SMSV or a related virus, if present in harp seals on the Canadian east coast, could spread to mink via seal meat.

Parapoxvirus infections have been reported in several pinniped species (Wilson et al., 1972; Simpson, et al., 1994). Lesions consist of small raised nodules that enlarge and become 1-3 cm in diameter (Wilson et al., 1972; Hicks and Worthy, 1987; Smith A. W., 1987 a; Simpson, et al., 1994). They often ulcerate and can become secondarily infected with bacteria. Histologically there is hyperkeratosis, large eosinophilic intracytoplasmic inclusion bodies in enlarged degenerating stratum spinosum cells, and there is often a marked mixed inflammatory cell infiltrate in the dermis (Hicks and Worthy, 1987). Orthopoxvirus has been isolated from pox-like lesions in grey seals (*Halichoerus grypus*) (Osterhaus et al., 1990). The pathogenic importance of the orthopoxvirus could not be determined since parapoxvirus particles were isolated from the same lesion. Poxviruses are often species-specific, but some interspecies transmission occurs. In an outbreak of poxvirus in captive gray seals, two people caring for the seals developed nodule-like lesions on their fingers that contained virus particles identical with those isolated from the seals (Hicks and Worthy, 1987). There is thus the possibility that mink could contract a poxvirus infection from infected seal meat.

Herpesvirus has been shown to cause mortality in harbor seals (*Phoca vitulina*) (Osterhaus et al., 1985). Clinical signs and pathologic changes were dominated by acute pneumonia, focal hepatitis and, in some cases, acute gingivostomatitis. Antibodies against herpes virus have also been found in harp seals but no clinical signs or pathological changes were redorded at the time of sampling (Stuen et al., 1994). Since different strains of herpesviruses can lead to different clinical manifestations in different species, a seal herpes virus might be contagious for mink.

CHAPTER 2

EPIDEMIOLOGY OF PODODERMATITIS IN FARMED MINK IN CANADA

2.0 Introduction

In the spring of 1996 an apparently new skin disease affecting the feet of breeder mink (*Mustela vison*) was reported by farmers in central and eastern Canada. Farmers observed swelling, ulceration, and thickening of the skin primarily on the plantar aspect of the feet. They also noted that affected mink were often hesitant to breed, presumably due to their painful feet. Occasional mink died with the condition but most mink appeared to recover uneventfully. Mink farmers associated the condition with the feeding of seal meat obtained from processors on the east coast of Canada.

During the later part of 1996 and the beginning of 1997 several mink were submitted for necropsy to the Ontario Veterinary College (OVC) and the Animal Health Laboratory, University of Guelph and to provincial veterinary diagnostic laboratories in Nova Scotia and New Brunswick (Ferns, 1997; Goltz, 1997). Superficial to deep ulcers were observed on the footpads, especially in junctional areas where the thick footpad joins the furred skin. The ulcers were often covered by scabs and debris. The dominant feature was hyperkeratosis of the pads and/or an area of the skin plantar to the metatarsal bones. In some cases the nail beds were infected, resulting in sloughing of toes. Occasionally, there were lesions on the nose pads, the corners of the eyes, and the head. Several bacterial species were isolated from the lesions, with *Staphylococcus* spp. dominating. Antibiotic treatment, in accordance with sensitivity tests, had limited success and new cases kept emerging. Although the disease was not widespread and did not cause high mortality, it did cause economic losses to a number of Canadian mink farmers, primarily through the loss of breeding males. In the spring of 1997 the Canada Mink Breeders'

Association approached OVC to investigate the disease and its possible association with feeding seal meat.

The first step in the investigation of mink footpad dermatitis was to attempt to determine how widespread the condition was and how common the use of seal meat was. The population at risk and the prevalence of disease were not known. A descriptive retrospective study, in the form of a mailed questionnaire, and a cross-sectional study of lesions and their host-specific distribution on selected farms were carried out in order to obtain information on the level and distribution of the disease as well as a possible association with feeding seal meat. A small study to determine if offspring from affected parents would contract the disease also was carried out.

2.1 Materials and Methods

2.1.1 Survey

A questionnaire was mailed to all mink farmers in Canada (n = 236) registered with the Canada Mink Breeders Association (CMBA). The survey form consisted of a cover letter containing a general description of the lesions that had been observed in necropsied mink with footpad dermatitis, and a questionnaire consisting of multiple choice questions, yes/no questions and a few questions that required numerical answers (Appendix 1). Questions were asked about number and color phase of breeding stock; type of housing; vaccination routines; use of prophylactic antibiotic treatments; occurrence of some common mink diseases; and type of feed used. Related to feed, questions were asked about whether seal meat had been a component of the diet during the previous year and, if so, in what concentration, in what form (raw or cooked) and during what months it had been used. Concerning pododermatitis, one question asked whether lesions similar to

those described had been observed in any mink during the last year. If the condition had been observed, farmers were asked to state what months of the year the condition had been observed; the color phase, gender and number of animals affected, as well as whether or not the animals had been treated with antibiotics. The perceived success rate of the treatment also was to be reported.

Prevalence proportions were calculated by dividing the number of animals reported to have lesions in an age and gender group by the total number of animals reported in that category. Average prevalences were calculated by weighting each farm's prevalence by its number of breeding mink.

2.1.2 Prevalence on Selected Farms

Six affected farms and three non-affected farms in southern Ontario were visited during the 1997 pelting season (November- December 1997) in order to record the prevalence of lesions among pelted mink. Farm selection was based on convenient location and on the presence or absence of lesions as determined by the survey.

A total of 547 males and 517 females were examined on the affected farms. A total of 180 mink were examined on three farms that had not seen the disease. The examined mink included black, mahogany, iris, pastel and demi-buff colored males and females. However, due to logistical problems and variations in the number of animals pelted per day per farm, the number of animals per age group, gender and color on each farm varied between 0 and 140 (Table 2.1). Mostly juvenile animals were being pelted at the time of observation, which resulted in the following age and gender distribution on the affected farms: 502 juvenile males, 442 juvenile females, 45 adult males, and 75 adult females.

Mink were killed with carbon monoxide (CO) and placed on cooling racks by farmers before the actual pelting procedure began. Between 20 and 40 mink were killed

at one time starting in a shed at one end of the farm and continuing through the sheds in order. Each batch of 20 to 40 mink was non-systematically placed on the cooling racks. A sampling interval was selected depending on the total number of animals to be pelted in a specific gender and age class that day. Due to different pelting practices and farm sizes, the interval varied from looking at every second animal laid on the rack to looking at every fourth animal laid on the rack. For every inspected mink the presence or absence of lesions on each foot, around the nose, and on other parts of the face was recorded. In addition, blood collection (for future analysis) was tried on every examined animal until 25-40 samples had been obtained.

In an attempt to examine more adult males and in order to increase the total number of animals examined, one affected farm was re-visited and two additional affected farms were visited after the breeding season in March 1998, the time when mink that will not be used in the next breeding season ("surplus" males and barren females) are culled. The age and gender distribution was: 288 juvenile males, 27 juvenile females, and 103 adult males (Table 2.2). Animals classified as juveniles were animals born the previous spring.

2.1.3 Susceptibility of offspring

A group of mink kits was inspected on each of two farms (A and B) shortly after weaning and on two additional occasions in the fall of 1998. Farm A had 5000 breeding females and 1000 breeding males. Seal meat had been fed as part of the food ration from September through November 1996. No mink with pododermatitis were reported in the survey sent out in 1997. Mink were bought in from an affected farm early in 1998. During the 1998 breeding season pododermatitis was noticed in many mink. Up to 71% (n=128) of juvenlie, mahogany male mink were affected when inspected in March 1998.

Farm B had 4500 breeding females and 800 breeding males. Seal meat had been fed as part of the food ration from April through December 1996. Pododermatitis was observed among breeding animals in February and March 1997. Lesions were then observed among young and adults sporadically throughout the year with an apparent increase among kits in June and July 1997 and again among breeding animals in February and March 1998. Inspection of mink during pelting in March 1998, showed that all inspected adult male iris mink (n=12) and all inspected juvenile male iris mink (n=44) were affected.

One or both parents of the kits inspected in the summer and fall of 1998 were known to have, or to have had, pododermatitis. None of the kits on either farm had been fed seal meat. On farm A, 87 mahogany kits (40 males and 47 females) were inspected on July 1, August 7 and October 28, 1998. At the time of the first inspection the kits were still housed with their mother. They were weaned a week later and housed two per cage in adjacent cages. On farm B, 40 iris kits (20 females and 20 males) were inspected on July 15, August 5 and October 6, 1998. The kits had already been weaned at the time of the first visit and were housed two per cage in adjacent cages. The presence or absence of foot lesions and the type of lesion was recorded.

2.1.4 Statistics

Associations between each combination of two categorical variables were analyzed using Pearson's Chi-square test. The Mantel-Hanzel Chi-square was used to control for farm effects. Interaction among farms was assessed using Yates interaction Chi-square (Norman and Streiner, 1994 a). Tables with expected frequencies <1 were not included and 2x2 tables with any cell containing "0" were not considered for analysis. Student's t-test was used to compare the difference between the means of the amount of seal meat fed

in affected and non-affected farms (Norman and Streiner, 1994 b). Data were analyzed using the computer software Statistix for Windows (© 1985, 98. Analytical Software, Tallahassee, FL).

2.2 Results

2.2.1 Survey

Completed surveys were returned by 81 out of 236 farmers (34%). Two respondents were no longer raising mink and six of the respondents reported their information in combination with that of a relative with whom they shared the farm. After these farms were excluded/combined, 73 farms remained for analysis. These farms reared a total of 147,180 female breeding mink and 24,850 male breeding mink. Farmers were not asked to report the number of juvenile animals on their farm. Survey results are summarized in Appendix 2.2.

Age, Gender and Color Distribution

Twenty-two out of 73 farms (30%) had seen lesions compatible with pododermatitis on their mink. Three out of the affected farms did not specify the number of mink with lesions. Upon follow-up by telephone, they indicated that many mink were afected but that they did not know how many. These farms were therefore not included in the prevalence calculation. Out of the 19 remaining farms, 11 reported lesions on adults only; seven reported lesions on adults and juveniles; and one reported lesions only on juveniles. The prevalence of mink with lesions consistent with foot pad dermatitis reported by farmers varied among the 19 farms in adult females from 0% to 3.23% with a weighted average of 0.25% and in adult males from 0% to 17.50%, with a weighted average of 6.26%. When data were combined for all farms, males were found to be

significantly more likely to have lesions than females ($p<0.01$). However, the strength of this association varied significantly among farms (interaction X^2, $p<0.01$). The condition was reported in a total of 1005 juveniles from eight out of the 19 farms. Prevalence among juveniles could not be calculated since the total number of juveniles per farm was unknown. However, if it was assumed that on average a female produces 4.5 kits, the prevalence of disease would range between 0% and 3.44% with a weighted average of 0.58% on affected farms. Lesions were reported in all color phases. It was not possible to compare prevalences in different colors because of inconsistency in the way in which color information was reported and because the total number of juveniles was not available.

Seal Meat Utilization

Out of the 73 responding farms, 40 had fed seal meat within the last year and 33 had never fed seal meat. Of those feeding seal meat 21 (52.5%) had seen foot pad lesions and 19 (47.5%) had not. Only one out of those who had not fed seal meat had noted foot lesions. However, this farm had only seen lesions after mink were bought from a farm where foot lesions were present. The association between feeding seal meat and the presence of lesions on the farm was significant ($p<0.01$). The proportion of the total feed ration that was composed of seal meat varied between 0.25% and 25% with an average of 10.65% on all farms feeding seal meat. Of the 40 farms feeding seal meat, there was no significant difference in the amount of seal meat used on those with lesions and those without lesions (11.06% and 10.25% respectively) ($p=0.67$). When seal meat was used in the ration it was always added as raw product. Most farmers mixed their own feed (86% and 74% respectively on farms with and without lesions) rather than buying a ready mix.

Seal meat was obtained from four main distributors. All the distributors were represented in both the affected and non-affected farms.

Temporal Distribution of Seal Meat Use and Presence of Disease

Between January 1996 and August 1997, seal meat was being fed on at least four farms (not necessarily the same four farms) throughout the year (Fig. 2.1). The largest number of affected and non-affected farms feeding seal meat at one time occurred in the period between June and November 1996. Disease was reported every month from at least two farms starting in August 1996, with the largest number of farms reporting disease in September-November 1996 and February-March 1997. None of the farms that had fed seal meat and had affected mink, reported seeing disease previous to the introduction of seal meat into the mink diet.

Antibiotics

Mink with lesions were treated with antibiotics on 18 out of 22 farms (including the farm that did not feed seal meat). Intramuscular injection with penicillin for 3-4 days was the most common treatment (15/18). Most farmers (11/15) reported that approximately half of the mink treated with penicillin recovered. Three farms reported very good recovery rate with penicillin treatment and one reported that it had no effect. Enrofloxacin was used with good results on one farm but only half of the mink thus treated recovered on another farm. Trimethoprim sulpha was used on two farms with about half of the mink recovering. One farm reported good results with erythromycin treatment. The four farms on which antibiotics were not administered also reported a recovery rate of approximately 50%. Regardless of treatment, most farmers commented that many mink that seemed to have recovered relapsed after some time.

Routine prophylactic use of antibiotics in the feed in periods of stress was reported by ten out of the 22 affected farms (45.5%) and by seven out of 14 unaffected farms (50%) that had fed seal meat. Five unaffected seal meat-feeding farms did not respond to the question. Similarly, seven out of 14 unaffected farms not feeding seal meat reported using prophylactic antibiotic treatments (18 did not respond to this question). At the farm level, there was no significant statistical association between the presence of lesions and the absence of prophylactic antibiotic use (p=0.79, n=36 seal meat feeders; p=0.75, n=50 total respondents).

Vaccination Routines and Disease Status

All farms except one reported vaccinating against botulism, distemper, and mink virus enteritis. The remaining farm vaccinated only against distemper. Thirty nine percent of all farms vaccinated against *Pseudomonas aeruginosa* pneumonia. There was no significant association between the absence of lesions on the farm and the use of pseudomonas vaccine either within farms feeding seal meat (p=0.73) or within all farms (p=0.26). The presence of lesions was not significantly associated with the reported sporadic presence of distemper (p=0.29 seal meat feeders; p=0.32 all farms) aleutian disease (p=0.58 seal meat feeders; p=0.93 all farms), tyrosinemia (p=0.45 seal meat feeders; p=0.41 all farms) or kidney disease (p=0.17 seal meat feeders; p=0.96 all farms).

Housing

Several types of caging and bedding were individually tested for an association with the presence of lesions. A farm was classified as using a particular type of caging or bedding if they used it at all. The occurrence of lesions was not found to be associated with any specific cage or bedding type, in farms feeding seal meat (all p-values>0.21) or in all farms (all p-values>0.23)

2.2.2 Prevalence on Selected Farms

During examination of mink at normal pelting time (November-December 1997) lesions were observed in one or more locations on 246 of 1064 mink (23%). The prevalence on each of the six farms varied between 6.3% and 69% in males and between 0.8% and 12% in females (Table 2.1). When combining data from all farms, regardless of farm size and the percent of animals examined within age, sex and color category per farm, lesions were significantly more prevalent among males than females. The higher prevalence in males was statistically significant (with all data combined, $p<0.001$). The significantly higher prevalence in males was also confirmed when analysis was restricted to data on juveniles on farms where both sexes were examined in specific color groups ($p<0.001$). The prevalence of mink with at least one lesion among black and mahogany colored juveniles was similar to that among pastel and demi colored juveniles. The highest prevalence was observed in juvenile iris colored males on one farm, which had a significantly ($p<0.001$) higher prevalence than juvenile black males on the same farm. No juvenile iris females were examined (Table 2.1). No lesions were observed on any of the mink ($n=180$) examined at the three visited farms that, according to the survey, did not have the disease.

The anatomic distribution of lesions on the mink showed a predilection for the hind feet. Of the affected animals examined ($n=246$), 79.2% had lesions on their left hind foot, 74.7% had lesions on their right hind foot, 6.9% had lesions on their left front foot, 11.6% had lesions on their right front foot, and 2.4% had lesions on their face.

The prevalence of affected mink identified in March 1998, when surplus animals were culled, was higher than the prevalence observed in November-December 1997. Prevalences in different colors and age groups varied between zero and 100% (Table 2.2).

No adult females were culled at this time. The prevalence of male mink with at least one lesion among black and mahogany colored juveniles was, as in November, similar to that among pastel and demi-buff colored juveniles. The highest prevalence was again observed in iris colored males. Only two female demi colored mink were examined.

As in November-December, lesions in March were most abundant on the hind feet. Of the affected animals examined (n=330), 95.2% had lesions on their left hind foot, 99.7% had lesions on their right hind foot, 26.4% had lesions on their left front foot, 28.8% had lesions on their right front foot, and 1.5% had lesions on their face.

2.2.3 Susceptibility of offspring

On farm A, no lesions were found on any of the 87 kits during the first visit. Five weeks later, 16 (18%) mink were affected. Of these, nine (10%) had small calluses in the metatarsal region and five (5.7%) had ulcers or crusts on one or more feet. Two (2.3%) had some crusting around the nose. The ulcerative lesions ranged from small (2-3 mm diameter) crusts on and around pads to large bleeding ulcers on the metatarsal area to partially healed ulcers with some scabs (up to 6-7 mm long). During the third inspection (7 weeks later), a total of 31 (36%) mink were affected. Of these, 28 (32%) had small calluses or healed lesions on pads or the metatarsal region, one (1.1%) had an ulcer (4mm diameter) on one pad and two (2.3%) had crusting around the nose.

On farm B, four out of the 40 mink (10%) had small crusts on their feet and two others (5%) had alopecia in the metatarsal region at the time of the first inspection. Three weeks later, 18 (45%) mink had lesions. Of those that had lesions at the first inspection, three were worse, two were unchanged and one had healed. There were six additional mink with sores and seven additional mink with scabs or hyperkeratosis of the plantar metatarsal region. At the last inspection time (four and a half weeks later), 29 (73%)

mink were affected. Thirteen had additional or more severe lesions than at the previous inspection and five had lesions that were healing. Eleven additional mink had either calluses or crusts on one or more feet.

2.3 Discussion

On-farm prevalence studies and results from a questionnaire sent out to mink farmers confirmed that pododermatitis was present among mink on a number of farms in Canada between 1996 and 1998. Many farmers perceived the pododermatitis to be a significant problem that could potentially lead to economic losses. This study attempted to establish the prevalence of the condition in different age, sex and color categories and to determine its association with some possible causal factors. All ages and colors seemed to be affected. Males were more often affected than females and the hind feet were more often affected than the front feet. The data supports an association between pododermatitis and the use of seal meat in the mink feed. No association was found between pododermatitis and the other possible causal factors studied.

The observed prevalences and the association with feeding seal meat could, however, be biased. The low response rate to the questionnaire (34%) is a potential source of bias. The bias results from potential differences between respondents and non-respondents in both measured variables and unmeasured confounders; the extent of the bias is unknown (Cowen et al., 1985). Ideally a response rate of at least 70% should be achieved in surveys of disease (Leech, 1971). However, data from questionnaires with low response rates (34-40%) that have been followed up by personal interviews have proven to be representative in some cases (Selby et al., 1973). In the present study, the true prevalence rates for all mink farms may differ from those reported by the farms that chose to

respond. It is possible that farmers that had experienced the disease on their farm were more likely to take the time to answer than those that had not. Another source of bias is the cover letter that accompanied the questionnaire which mentioned the possibility of a link existing between seal meat and pododermatitis. This information could have affected the way in which farmers responded or chose not to respond. Nonetheless, the responses did provide an indication that footpad dermatitis was present on at least 22 farms and that it was linked to the use of seal meat.

Both the survey and the farm visits at pelting time suggested that all color phases were affected and that males had a higher prevalence than females. However, the prevalence observed at pelting time was higher than that reported in the surveys. The greater prevalence seen at pelting time could be a reflection of the disease spreading, since the survey was conducted four months prior to pelting time, or it could be the result of farmers not detecting some of the affected mink. In most of the farms where mink were inspected at pelting time, farmers were surprised at the large number of affected animals, suggesting that many mink probably had not shown any detectable signs of disease. It is, therefore, probable that the farmers underestimated the number of affected animals when they completed the questionnaires. Furthermore, in some cases it may be difficult to differentiate the pododermatitis from other foot lesions, which could either increase or decrease the reported prevalence.

The prevalence of disease among juveniles could not be calculated from the survey data since the total number of juveniles per farm was not available. The prevalence among juveniles during pelting time seemed to exceed that among adults. This may reflect a spread of the disease to the younger generation, healing in the older animals or greater susceptibility in the young. The slightly higher prevalence in the iris colored

animals may be related to them being homozygous for the recessive aleutian gene. The aleutian gene is responsible for the "blue" color phases in mink but is also associated with the Chediak-Higashi Syndrome (CHS). Mink with CHS have deficient leukocytes which make them less efficient at coping with bacterial diseases (Prieur, 1996). However, iris colored animals were only observed on one farm, making it difficult to extrapolate these findings to the mink population at large. At the present time it is unknown why more males than females are affected and why the hind feet are more often affected than the front feet. It is possible that the hind feet are preferentially affected because mink often stand upright in their cages and put all their weight on their hind feet. Males may be preferentially affected due to larger body weights.

The increased prevalence observed in pelted mink in March 1998 compared to November-December 1997 may reflect a spread of the disease. Higher prevalences among both adults and juveniles (animals born the previous spring and bred the present year) may also represent selective culling of males that did not perform well during the breeding season, perhaps related to sore feet. The high prevalence observed in pelted mink in March 1998 and the slightly higher prevalence of disease reported in the survey for October-November and February-March may reflect times of increased stress for the mink. The late fall is the time when mink are being primed for pelting and have increased metabolic requirements associated with rapid body growth and fur development. The late winter/early spring is the time when they are getting ready to breed. Males are excessively active and pace the cages, perhaps increasing the risk of traumatic injuries to the feet. However, it is more likely that the greater number of affected mink in these months is artefactual and just reflects the increased handling and inspection of mink at these times.

There is a strong statistical association between the disease and feeding seal meat but the survey's bias must be taken into account. Of those mink farmers answering the survey, only those that had fed seal meat at some point or had bought in animals from an affected farm reported the disease. No farm reported seeing the disease before the use of seal meat. These facts would support an association with seal meat. However, the fact that almost half of the farmers who used seal meat did not report lesions and the fact that there was no difference in the way in which seal meat was utilized on farms with and without lesions argues against the causal role of seal meat. If seal meat is the "source" of pododermatitis, it would appear that only certain seal meat batches contain the etiologic agent, or that other predisposing factors are involved. Furthermore, the failure to report use of seal meat before lesions were observed may be due to the unclear wording of the survey questions. Survey questions referred to "the last year" which was interpreted by most farmers to mean the last 12 months but by some to mean the last calendar year.

The association of pododermatitis with seal meat is further supported by historical evidence that suggests that seal meat has the potential to cause infection and disease in mink. Dr. Austin Larsen of the Utah fur co-op, U.S.A., noted that in the early 1970's mink that were being fed seal meat from the west coast of the U.S.A. developed footpad lesions that were unresponsive to antibiotics (Larsen, 1997). The farmers stopped feeding seal meat and the condition eventually disappeared. There are, however, no published reports on this condition. In 1974 Sawyer et al. (1978) observed that 2% of northern fur seals harvested around St Paul Island, Alaska had vesicular lesions on their flippers. The seals were being harvested for pelts and subsequently processed for use as mink feed. Eight seal carcasses with vesicular lesions were ground-up and examined for the presence of viruses. San Miguel sea lion virus (SMSV), a calicivirus that has been isolated from

several species of marine mammals off the western coast of North America, was isolated from four of the eight carcasses.

Infection with SMSV produces vesicular lesions on the flippers of marine mammals and vesicular lesions indistinguishable from those of vesicular exanthema in swine (Smith and Boyt, 1990). Since potentially infected seal meat was being used as mink feed, mink were experimentally fed seal meat infected with San Miguel sea lion virus (Wilder and Dardiri, 1978). The mink became infected (produced antibodies and virus could be isolated), but clinical signs were inapparent. It is thus possible that SMSV or a similar virus could potentially spread to mink via seal meat.

The data from our study further suggests that pododermatitis can spread between mink. The affected farm that had not fed seal meat did not observe the problem until after animals from an affected farm were introduced. One of the seal meat-feeding farms that originally reported not seeing pododermatitis, became affected later during the study period after buying mink from an affected farm. Most juveniles inspected had never eaten seal meat since many farmers stopped using it before these animals were weaned. Nonetheless, a large number of juveniles were affected. When offspring from parents that had been affected were followed for 11 to 12 weeks after weaning, small lesions were noted on up to 36% and 73% of the kits on two farms. Certain viruses, such as feline calicivirus (FCV), persist as an active asymptomatic infection in recovered animals (Pedersen, 1987). Clinically healthy cats can actively shed varying amounts of FCV in the saliva for many months (Wardley, 1976; Wardley and Povey, 1976). Kittens can become infected as young as 4-8 weeks of age and develop clinical signs when maternal antibodies decline. Maternal antibodies have a half life of 15 days and can persist until ten to 14 weeks of age (Johnson and Povey, 1983). If the cause of mink pododermatitis

was viral, it is thus possible that adult female mink could infect kits before weaning and that kits could develop lesions later. The lesions could heal more or less rapidly depending on whether or not they were secondarily infected by bacteria. Therefore, although seal meat could have been the original source of the disease, it does not seem to be essential for its propagation.

Foot lesions in mink previously have been described in association with hereditary tyrosinemia (Christensen et al., 1979), urolithiasis (Tomlinson et al., 1982) and canine distemper (Budd et al., 1966). No association was found between pododermatitis and either the occurrence of these conditions on farms or with vaccination routines. This strengthens the theory that the pododermatitis described is a new condition affecting mink. Bacteria are commonly associated with pododermatitis in dogs (Muller et al., 1989). It is not known whether the bacteria that have been isolated from submitted mink cases play a causal role or are simply secondary to another etiologic agent, such as a virus. A secondary role could explain the reported poor effect of antibiotics. Alternatively, there may have been poor penetration of antibiotics into the footpads, delayed onset of treatment, inappropriate dosage or too short a treatment period.

Since the mink had lesions on the underside of the feet, it was of interest to consider the type of cage and bedding material they walked on. Housing was not an important causal factor since all farms, except one, used standard wire pens. The most common bedding materials used were straw and shavings. No association was found between the presence of lesions and the use of straw, shavings or other bedding material (excelsior and beet pulp). The type of bedding could, however, in certain cases have exacerbated the lesions. For example, shavings easily adhere to small ulcers.

When considering the negative results from this study, it is important to keep in mind that the power of the study is low due to the small sample size and the lack of variability in several of the hypothesized risk factors. In particular, when the inspected mink were divided into color, age and sex categories, the number per category per farm was often small. The response rate to the questionnaire was low and may have been improved with a follow-up telephone interview. This may also have helped clarify some of the questions and perhaps increased the reliability of the results. The study does, however, provide a base for continued research on the epidemiology and etiology of mink pododermatitis.

2.4 Conclusion

Information obtained from a retrospective study in the form of a questionnaire, observations of mink at pelting time, and necropsies suggest that footpad dermatitis is an emerging disease in farmed mink in Canada. Mink farmers have not seen these types of lesions until recently and the type of lesions observed have not been described previously in the literature. All color phases are affected and the condition seems more prevalent among males than females. The condition appears to spread within farms and between farms when live animals are transferred, suggesting an infectious etiology. There is a possible association between feeding seal meat to mink and the onset of the disease. No association was found between the disease and any other variables investigated (housing, vaccination routines, other diseases, and antibiotic use). To further characterize the disease it is necessary to establish the way in which it spreads, possible etiologies (including non-infectious etiologies), and whether or not harp seals on the east coast of Canada carry any diseases that are potentially harmful to mink.

Table 2.1 Prevalence (%) of foot and/or face lesions and number of mink examined (in paraentheses) on six affected farms (1-6) and three non-affected farms (7-9) in Ontario during pelting, November/December 1997.

	Black/Mahogany				Pastel/demi			Iris	
	Adult		Juvenile		Adult	Juvenile		Juvenile	
	Male	Female	Male	Female	Female	Male	Female	Male	Female
Farm 1	NA[§]	NA	6.3 (80)	4 (50)	NA	NA	NA	NA	NA
Farm 2	NA	NA	36 (120)	7.1 (140)	NA	NA	NA	69(133)	NA
Farm 3	18(45)	NA	NA	NA	NA	37 (84)	4 (71)	NA	NA
Farm 4	NA	6.7 (75)	53 (57)	NA	NA	NA	NA	NA	NA
Farm 5	NA	NA	36 (28)	12 (51)	NA	NA	NA	NA	NA
Farm 6	NA	NA	NA	0.8 (130)	NA	NA	NA	NA	NA
Mean* 1-6	18(45)	6.7 (75)	31(285)	5.1 (371)	NA	37 (84)	4 (71)	69(133)	NA
Farm 7	NA	NA	0 (60)	NA	NA	NA	NA	NA	NA
Farm 8	NA	NA	0 (2)	0 (33)	0 (1)	NA	0 (9)	NA	0 (15)
Farm 9	NA	NA	NA	0 (40)	NA	NA	0 (20)	NA	NA
Mean 7-9	NA	NA	0 (62)	0 (73)	0 (1)	NA	0 (29)	NA	0 (15)

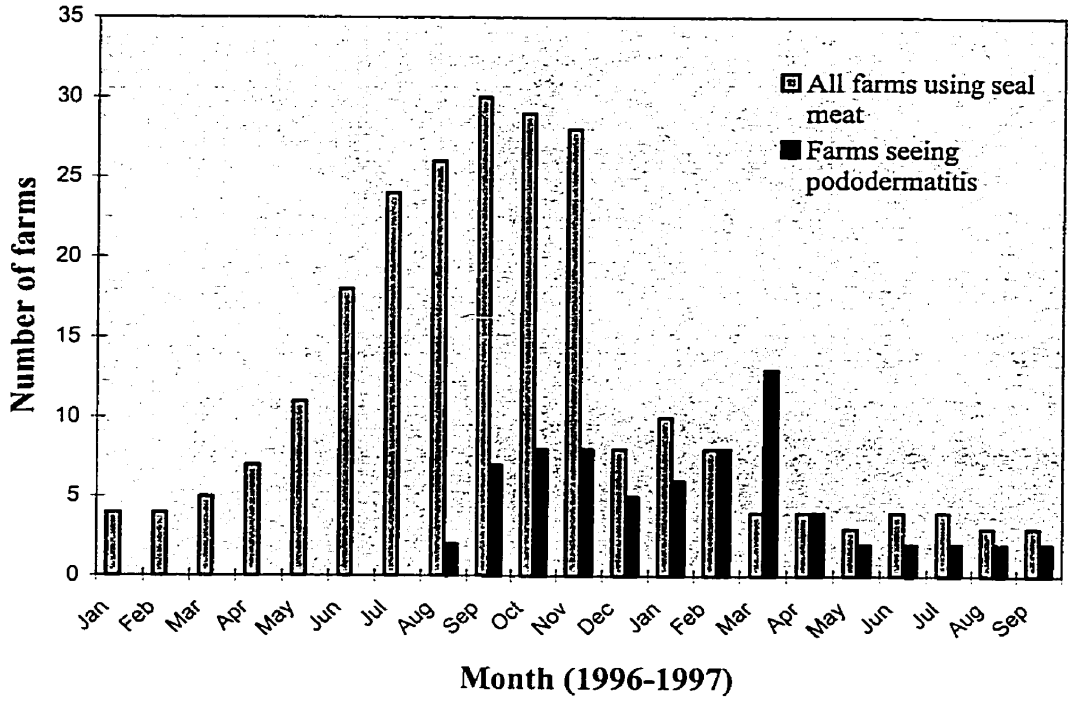
[§] NA: Not applicable. Mink in this category were not inspected or were not present on the farm.
*Mean: weighted mean.

Table 2.2 Prevalence (%) of foot and/or face lesions and number of mink examined (in parentheses) on three affected farms in Ontario during pelting, March 1998.

	Black/Mahogany			Pastel/demi		Iris	
	Adult	Juvenile		Juvenile		Adult	Juvenile
	Male	Male	Female	Male	Female	Male	Male
Farm 1	NA[§]	71 (128)	NA	50 (4)	NA	NA	NA
Farm 2	87 (86)	87 (63)	NA	NA	NA	100 (17)	100 (44)
Farm 3	NA	84 (44)	16 (25)	100 (5)	0 (2)	NA	NA
Mean* 1-3	87 (86)	78 (235)	16 (25)	78 (9)	0 (2)	100 (17)	100 (44)

[§] NA: Not applicable. Mink in this category were not inspected or were not present on the farm.
*Mean: weighted mean

Fig. 2.1 Temporal distribution of seal meat use and presence of pododeratitis on mink farms between January 1996 and September 1997.



CHAPTER 3

PODODERMATITIS IN FARMED MINK IN CANADA: GROSS AND HISTOLOGIC DESCRIPTION

3.0 Introduction

In the spring of 1996, mink farmers in central and eastern Canada reported the occurrence of a "new" skin disease of mink (*Mustela vison*) affecting the foot pads and facial skin. The lesions were described as ulcerated, crusty areas of thickened skin affecting the margins of the foot pads, the nail beds and occasionally the nose pads, eyelids and other areas of the head. Male mink were observed with lesions more frequently than females. Morbidity rates were variable among farms and mortality rates were low. The main economic impact was that affected male mink failed to breed, presumably because of the painful foot pad lesions. Farmers suspected that the disease was in some way linked to the feeding of meat from harp seals (*Phoca groenlandica*) harvested off the east coast of Canada.

The initial mink examined at the Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada had severely swollen feet with ulcerated foot pads and deep pyodermas. The lesions resembled deep-seated bacterial infections. A variety of bacteria including *Staphylococcus* sp., *Streptococcus* sp., *E. coli* and other organisms were cultured from several animals, but no distinct pattern emerged. An epidemiologic study showed that all color phases present on the farms (standard dark, mahogany, pastel, blue iris, demi-buff) were affected, that the condition seemed to spread within and between farms, and that there was no association between the disease and types of housing,

bedding material, vaccination routines, use of antibiotics or other diseases present on the farms (Bröjer, 2000, Ch.2).

In order to obtain information on the initial insult and the progression of the lesions, we attempted to find mink with early lesions. This was difficult as mink farmers do not routinely handle their mink except at specific times such as breeding, weaning, grading and pelting. During other times of the year, farmers will only notice diseased mink if they stop eating, change their behavior significantly or if blood or other discharge is observed on the wire of the cage. Only a few mink with early lesions were found at pelting time even though we inspected large numbers of animals.

This study was designed to examine mink on six affected farms at breeding, weaning and pelting time, in an attempt to identify early cases of pododermatitis and to describe the spectrum of macroscopic and microscopic lesions associated with this condition.

3.1 Materials and Methods

Gross evaluation of lesions was carried out by inspecting live mink on six affected farms at various times between July 1997 and December 1998 (n= 130) and by inspecting dead mink at pelting time in December 1997 (n= 1471). Complete necropsies were performed on 59 mink. Of the 59, fourteen mink carcasses with foot lesions were submitted by farmers, 19 mink were taken live from farms during the on-farm inspections and euthanized with sodium pentobarbital in propylene glycol (Euthanyl forte, MTC Pharamaceuticals, Cambridge Ontario, N3C 2W4) (University of Guelph Animal Utilization Protocol 98R040), and 26 carcasses were collected at pelting time. Samples of brain, heart, lung, trachea, spleen, liver, kidney, bladder, tongue, stomach, duodenum,

small intestine, colon, main mesenteric lymphnode, thyroid gland, and affected skin parts were placed in 10% buffered formalin. In addition, 13 affected feet were collected at pelting time and placed in buffered formalin. The tissues were later trimmed, embedded in paraffin, sectioned at 6 micrometers, and stained with hematoxylin and eosin (H&E) for microscopic evaluation. Selected tissues were also stained with Periodic Acid Schiff (PAS), Warthin-Starry, Brown and Brenn, and Ziehl-Neelsen stains. Pieces of brain, lung, spleen, liver, kidney, small intestine, colon, and affected skin were frozen at -70°C for future analysis.

A total of 104 feet and seven face lesions were selected for both macro- and microscopic evaluation. The gross lesions were classified as alopecia and/or thickened skin; ulcers; ulcers with crusts and/or hyperkeratosis; and callosities or healed lesions. In addition, five feet without gross lesions were evaluated histologically. Each case was evaluated microscopically to determine the presence or absence of different follicular inflammatory patterns using Gross et al.'s (1997) anatomical classification of folliculitis and Caswell et al.'s (1995) classification of histopathologic lesions of canine demodicosis. In addition to hair follicle lesions, the presence of hyperkeratosis and dermal inflammation without involvement of hair follicles were noted. Each case was categorized according to the presence or absence of the following: hyperkeratosis, perifolliculitis, mural folliculitis, folliculitis, furunculosis, and dermal inflammation. Gross et al. (1997) define perifolliculitis as inflammation of the area around the hair follicle and inflammation that remains perivascular without involvement of the follicular wall. Mural folliculitis is defined as an inflammation which targets the follicular wall, primarily the outer root sheath at and above the level of the follicular isthmus and does not involve the pilar canal. Folliculitis resembles mural folliculitis but is characterized by

intraluminal accumulation of inflammatory cells. Furunculosis is a folliculitis which has resulted in the destruction of the hair follicle and is associated with a severe inflammatory reaction. Dermal inflammation is a diffuse dermal inflammation of varying severity which does not involve hair follicles.

Certain skin sections were found to contain particles that resembled eosinophilic inclusion bodies, and some lung sections had numerous large macrophages with phagocytized material (see results). In order to screen for virus particles and to evaluate the contents of the lung macrophages, 13 skin sections and five lung sections were processed for electron microscopic evaluation. The tissues were placed in 2.5% phosphate-buffered glutaraldehyde fixative, washed in phosphate buffer, post-fixed in 2% osmic acid in phosphate buffer, dehydrated in increasing concentrations of ethanol, and embedded in Epon. Sections one micrometer thick were cut and stained with toluidine blue, and selected areas were then sectioned at 60-90 nanometers with a diamond knife and viewed with an electron microscope (Japanese Electron Optics Limited 100S, JEOL 100S).

3.2 Results

Macroscopic lesions

Foot pad lesions were found in both male and female mink, and adults and juveniles of all color phases present on the farms. Affected mink had circular to elongated ulcers, ranging in size from 2-3 mm to 2 cm in diameter. The lesions had varying degrees of crusting, hyperkeratosis, necrosis, and lymphangitis, affecting primarily the feet. Lesions were most often observed on the plantar surface of the metatarsal region, volar aspect of the metacarpal region, junctional areas where foot pads

join the furred skin on both front and hind feet, and occasionally around nail beds. Less commonly, lesions were seen on nose pads, corners of the eyes, mucocutaneous junctions of the mouth, and sides of the face. Of the 104 feet with lesions chosen for macroscopic and microscopic evaluation, 30 were classified as having alopecia and/or thickened skin, 18 as ulcers, 39 as ulcers with crusts and/or hyperkeratosis and 17 as callosities or healed lesions. Three of the seven selected face lesions were classified as having alopecia and/or thickened skin, and four as ulcers with crusts and/or hyperkeratosis.

The small number and uncertainty in our ability to identify early lesions, made it difficult to formulate an accurate description of the progression of macroscopic changes. The earliest lesion apparent on gross examination was loss of hair (alopecia) and thickening of the skin on the plantar surface of the metatarsal region as well as small, yellow, hyperkeratotic strands on the edges of the main foot pads.

Some mink had thickened skin around and between toes which resembled thick-walled blisters, but no fluid could be withdrawn from them. Occasionally the plantar side of the foot was swollen, alopecic and had a small amount of clear exudate on the skin (Figure 3.1a). Although the epidermal surface appeared almost intact, on the cut surface a very small fistula was apparent (Figure 3.1b). In other cases small ulcers were noted in the epidermis, which on cut surface, was seen to connect to a fistula.

The more commonly observed lesions were circular to elongated ulcers, usually caudal to the main pad of the foot, or severe hyperkeratosis with ulcers and crusts (Figure 3.2). Ulcers also were seen frequently on the sides of toes and around the nail bed (Figure 3.3). Occasionally the lesions were severe and led to exudation and crusting of the entire underside of the foot (Figure 3.4). In other cases lymphangitis of the affected leg was observed.

In many mink the lesions had healed leaving a callus-like nodule. Several of the 1471 mink inspected during pelting had small calluses on the plantar metatarsal region which were difficult to classify as healed pododermatitis lesions or as callosities obtained from pressure or friction from the cages. These were not considered as pododermatitis cases.

Lesions in the face ranged from various amounts of gray-brown exudate around the nose and eyes to large crusts covering parts of the nose and eyes (Figure 3.5). The crusts were mostly superficial and could be removed easily. Since it is not uncommon for bite wounds in mink to become infected and crusty, mink that solely had crusts in the skin on other parts of the body were not considered to be affected by the pododermatitis "syndrome".

Internal organs of the 59 necropsied mink had few and mild macroscopic lesions per animal (Table 3.1). The lungs were congested and/or mottled dark red and tan in 53 % of examined mink. Splenomegaly was observed in 42% of the mink. The liver was either congested or pale and friable in some mink. The kidneys were pale and friable in 5% of mink and had a pitted subcapsular surface in 10%. The regional lymph nodes draining the skin lesions (primarily the popliteal lymph nodes) occasionally were enlarged. In 24% of the cases there was an increased amount of mucus in the mouth and/or nose. Other incidental findings included purulent pneumonia and pyothorax in one mink, subcapsular hepatic hematoma due to rupture of a fatty liver in one mink, and stomatitis due to a fish bone lodged between the upper canine teeth in one mink.

Microscopic lesions

Skin

The histologic findings were a continuum of increasing degrees of hyperkeratosis, follicular inflammation and dermal inflammation (Table 3.2). Sections from the foot pads of five normal, unaffected feet had no microscopic lesions except for mild hyperkeratosis in two animals. Skin from feet classified as alopecic or thickened (n=30) had the mildest microscopic changes. The dominant feature was orthokeratotic epidermal hyperkeratosis. Follicular plugging and hyperkeratosis was evident and in some cases there was a pale zone under the keratin layer (Figure 3.6). In more than half of these cases, there were discrete areas of perifollicular lymphocytic infiltration (Figure 3.7). Mild mural folliculitis was also present in 43% of the sections. Furunculosis and dermal inflammation were seen only occasionally in this group.

The histologic appearance of feet with ulcers (n=18) varied, depending on the degree of inflammation of the ulcers. There was partial to complete epidermal ulceration and many sections contained gram-positive cocci on the surface. In the non-ulcerated areas spongiosis, intracellular edema, and microabscesses occurred. All skin sections in this group had moderate to marked hyperkeratosis, perifolliculitis and mural folliculitis (Figure 3.8, Table 3.2). Several cases had dermal inflammation, folliculitis and furunculosis. The follicular inflammation was mostly lymphocytic but had a neutrophilic component near ulcerated areas. The dermal inflammation was primarily neutrophilic and was concentrated to the area around the ulcer. Regions with furunculosis contained necrotic cells, keratin, neutrophils, macrophages and occasional giant cells.

Feet with severe ulcerations, hyperkeratosis and exudation (n=39) were similar to those of ulcerated feet but with a more marked suppurative inflammatory response.

There was marked neutrophilic and lymphocytic infiltration of the dermis around the ulcer, marked perifolliculitis and moderate mural folliculitis and furunculosis (Figure 3.9, Table 3.2). There were many microabscesses present in the epidermis. There also was moderate disorganization of the basal layer and pigmentary incontinence.

Feet grossly classified as having callosities or healed lesions (n=17), histologically had acanthosis, hyperkeratosis and mild to moderate perifolliculitis and mural folliculitis (Table 3.2). A diffuse dermal inflammation was present in more than half of the cases and some of these also had a mild furunculosis. The perifolliculitis, mural folliculitis, and dermal inflammation consisted mostly of lymphocytes and plasma cells whereas the furunculosis had a mixed cell population with neutrophils, lymphocytes, and macrophages. Many of the hair follicles had a thickened outer root sheath, and the follicular area appeared larger than normal. In the areas with folliculitis the basal cells were often injured and there was pigmentary incontinence. The face lesions were similar to the foot lesions in the same macroscopic category.

In 20 cases (34%) there were eosinophilic droplets of variable size in the cytoplasm of stratum spinosum cells. These droplets displaced the nucleus to one side and thus resembled inclusion bodies. When examined under the electron microscope the epidermal inclusions were either droplets of keratin or empty vacuoles. Gram stain (Brown and Brenn stain) revealed gram positive cocci and occasional other bacteria on the surface of ulcerated sections. No fungi, spirochetes, acid fast bacteria or other organisms were found in sections stained with other special stains (PAS, Warthin-Starry, Ziehl-Neelsen).

Internal Organs

In accordance with the macroscopic findings, histologic evaluation of internal organs revealed a limited number of changes (Table 3.1). Close to half of the mink lungs were congested and had prominent accumulations of macrophages. Many of the macrophages were foamy macrophages consistent with alveolar histiocytosis (Dungworth, 1993), which is a common histologic finding in clinically normal mink. Other pulmonary macrophages differed from the foamy macrophages in that they contained an amorphous, eosinophilic (H&E stain) and mildly PAS-positive material in their cytoplasm. When viewed under the electron microscope the material in the lung macrophages was a non-specific, amorphous material with no specific structure and no resemblance to a potential pathogen. In 13 of the lungs with these macrophage accumulations, there were mild perivascular and/or peribronchiolar accumulations of mononuclear cells, primarily lymphocytes. Although many mink had splenomegaly, 25% had atrophic, depleted follicles and only two spleens had active germinal centers. Amyloid was found in and around splenic follicles in four cases. A similar homogeneous, eosinophilic material was present in nine additional mink but no special stains to confirm the presence of amyloid were carried out on those cases. Six spleens were markedly congested and one spleen showed splenitis with large numbers of coccoid bacteria and large numbers of neutrophils around the follicles. More livers were congested and had hepatocellular lipidosis than was expected from the gross appearance. Multifocal accumulations of plasma cells and lymphocytes, suggestive of Aleutian disease, were found in the portal areas of 7% of livers. The kidneys with gross pitting of the subcapsular surface had interstitial fibrosis and plasma cell accumulations suggestive

of Aleutian disease. The kidneys from 15% of the mink had lipidosis of tubular epithelium.

3.3 Discussion

The macroscopic lesions observed in mink with pododermatitis were non-specific. The spectrum of foot pad lesions ranged from a mild skin thickening and localized alopecia to extensive ulceration, hyperkeratosis and deep pyodermas. Many of the animals examined at pelting time had healed pad lesions. It was often difficult to determine whether certain callosities were healed lesions or a "normal" consequence of walking on wire cages. Microscopically, the lesions ranged from mild-moderate hyperkeratosis to peri- and mural folliculitis to ulcerations with furunculosis and extensive dermal inflammation.

Based on the mild microscopic changes, the lesions grossly classified as alopecia and thickened skin were assumed to represent the earliest stages of the disease. The mildest histologic picture was dominated by epidermal and follicular hyperkeratosis and mild to moderate perifolliculitis and mural folliculitis. In mink hyperkeratosis has mainly been associated with canine distemper (Pearson and Gorham, 1987). Diffuse orthokeratotic hyperkeratosis in dogs has, among other things, been associated with endocrinopathies (Scott, 1982) and nutritional deficiencies (Yager and Scott, 1993). Endocrinopathies are unlikely causes of the foot pad hyperkeratosis since the mink did not have any other clinical or microscopic signs consistent with hormonal disturbances. Nutritional deficiencies sometimes occur in farmed mink but are probably not the cause of hyperkeratosis since mink on several farms with different food rations began developing similar lesions during a short time span. The only factor in common for most

of the affected farms was the use of seal meat as a component in the diet (Bröjer, 2000, Ch. 2). However, many of the affected mink were kits that had not been fed seal meat. There are few published results from feed trials in mink using seal meat as part of the feed ration. A Norwegian study in which ensiled seal meat was fed to mink showed no negative effect on reproduction, growth or skin quality (Skrede, 1983).

Diffuse parakeratotic hyperkeratosis in dogs is suggestive of zinc-responsive dermatoses (Sanecki et al., 1982), dermatophytosis (Muller et al., 1989), some vitamin-A responsive dermatoses (Ihrke and Goldschmidt, 1983) and thallium poisoning (Zook and Gilmore, 1967). The zone of pallor observed beneath the keratin layer in some of the mink cases indicates inappropriate maturation or damage to keratinocytes (dyskeratosis) and also can be seen in zinc deficiency, certain metabolic deficiencies and thallium poisoning (Yager and Scott, 1993). There was no epidemiologic evidence to support a metabolic deficiency or an intoxication as a cause of the disease. It is possible that the seal meat included in the food ration contained substances that inhibited the uptake of zinc or affected the minks' metabolism in some other way. As mentioned above there are, to the best of our knowledge, no published feed trials supporting this theory. Skrede (1983) also mentioned that seal meat has a high concentration of Vitamin A. Thallium, an ingredient in older types of rodenticides, is unlikely to have beeen present on several farms in such a way that mink had access to it. In cases of thallium toxicity affected animals often show signs of systemic disease (Zook and Gilmore, 1967) which was not the case with the mink. Localized hyperkeratosis is also a common, non-diagnostic finding of many chronic dermatoses.

The lymphocytic peri- and mural folliculitis with basal cell injury and pigmentary incontinence, which was seen in addition to hyperkeratosis in many of the cases,

resembles conditions caused by a cell-mediated immune response. Lymphocytic mural folliculitis is, for example, seen in demodicosis (Caswell et al., 1995), in auto-immune mediated diseases such as pemphigus foliaceus (Ihrke et al., 1985) and in dermatophytosis (DeBoer and Moriello, 1995). Neither *Demodex* mites nor dermatophytes were detected in any of the sections. In pemphigus foliaceus discrete pustules are often seen within the wall of the superficial hair follicle (Gross et al., 1997) which was not the case in the mink. It is improbable that a large number of mink on different farms begin developing auto-immune mediated diseases within the same year.

Folliculitis and furunculosis was a common microscopic feature in mink that grossly had ulcers, crusts and hyperkeratosis. This is the most commonly recognized pattern of follicular inflammation and usually is caused by fungi, parasites or bacteria (Gross et al., 1997). As noted above, no fungi or parasites were identified in the sections despite the use of special stains (PAS, Warthin-Starry). Bacteria, mostly Gram positive cocci, were identified microscopically in many skin sections with ulcers. A variety of bacteria were also isolated from these skin sections with a predominance of staphylococci, but no clear cut pattern was seen (Bröjer, 2000, Ch. 4). The limited number of early lesions with folliculitis and bacteria, the presence of bacteria in the more severe cases and the variety of bacterial species isolated, indicates that the bacteria are likely secondary to a predisposing insult.

The blister-like thickening of skin seen around some toes, the intracytoplasmic eosinophilic inclusions in the spinous cell layer and the mural folliculitis, made us initially consider viruses as possible etiologic agents. Viruses such as pox (Bennett et al., 1985), herpes (Flecknell et al., 1979), distemper (Crook et al., 1958) and calici (Cooper and Sabine, 1972), which are either exclusively epitheliotropic or are systemic viruses

that also have an affinity for skin, have been associated with skin lesions in dogs and/or cats. These viruses could potentially infect mink and elicit an inflammatory response themselves or result in lesions that function as a port of entry for bacteria. If the virus caused small enough lesions, which secondarily became infected with bacteria, this could help explain the difficulty encountered in trying to detect early pododermatitis lesions. No virus particles were found on EM examination, but we cannot rule out the possibility that the sections we examined came from animals where the virus particles were no longer present.

The lesions found in internal organs were generally non-specific. Alveolar histiocytosis has been described in laboratory rodents, occasionally in cats and rarely in dogs (Dungworth, 1993). It also has been described in opossums (Brown, 1988), raccoons (Hamir et al., 1996) and llamas (Hamir et al., 1997). The cause of alveolar histiocytosis is unknown but may be related to bronchiolar obstruction, inhalation of particulate dust, disturbance of lipid metabolism or may be present without apparent cause (Brown, 1988). The accumulation of amyloid in some spleens could be idiopathic or could be related to the presence of a chronic infection such as the pododermatitis. Prolonged exposure to endotoxins can also lead to amyloid deposition. Experimental subcutaneous injections of *E. coli* endotoxin, for example, led to the deposition of amyloid in the spleen and liver of mink (Nordstoga, 1972).

The pododermatitis thus seemed to begin with a small lesion on the underside of the feet. The skin became thickened, ulcerated and was probably secondarily infected with bacteria. Microscopically, the lesions varied from hyperkeratosis and mild perifolliculitis to ulcerations with marked furunculosis and dermal inflammation. The lesions were non-specific but were consistent with an infectious etiology. The lymphocytic peri- and mural

folliculitis were probably reactions to an agent foreign to the body. However, the only agents identified microscopically were superficial bacterial colonies which would not be expected to give this type of lymphocytic reaction. Skin disease represents a disruption of the normal equilibrium that exists between the skin and its microflora or a failure of the host's defense response (Mason, et al., 1996). This can result in the overgrowth of commensals such as bacteria. Further investigations of the progression of the early lesions are necessary to discover the factors disrupting the skin's normal microbial balance and to further explore the mechanism behind mink pododermatitis.

Table 3.1. Macroscopic and microscopic lesions observed in internal organs of 59 mink with pododermatitis.

Organ	Macroscopic change and percent affected (n=59)		Microscopic change and percent affected (n=59)	
Lung	Congested/mottled	53%	Alveolar histiocytosis	46%
			Congested	42%
			Peribronchial/perivascular lymphocytic infiltrates	22%
Spleen	Enlarged	42%	Atrophic	25%
			Amyloid	22%
			Congested	10%
			Acute splenitis	2%
Liver	Congested/friable	20%	Congested	29%
			Fatty degeneration	19%
			Plasmacytosis	7%
Kidneys	Pitted surface	10%	Fatty degeneration	15%
	Pale and friable	5%	Plasmacytosis	8%
Lymphnodes	Enlarged/congested	29%	Reactive/congested	20%
Mouth/Nose	Mucus	24%	Not inspected	

Table 3.2. Prevalence of microscopic lesions in five normal mink feet and 104 mink feet with pododermatitis categorized according to their macroscopic appearance.

	Normal (n=5)	Alopecia or thickened skin (n=30)	Ulcers (n=18)	Ulcers with hyperkeratosis (n=39)	Callosity or healed lesion (n=17)
Hyperkeratosis	40%	97%	100%	100%	94%
Perifolliculitis	0%	57%	100%	100%	88%
Mural folliculitis	0%	43%	100%	98%	70%
Folliculitis	0%	7%	39%	82%	12%
Furunculosis	0%	20%	67%	82%	35%
Dermal inflammation	0%	7%	83%	100%	59%

Fig. 3.1a Swollen mink foot with alopecia and exudate on apparently intact epidermis in an area behind the main pad.

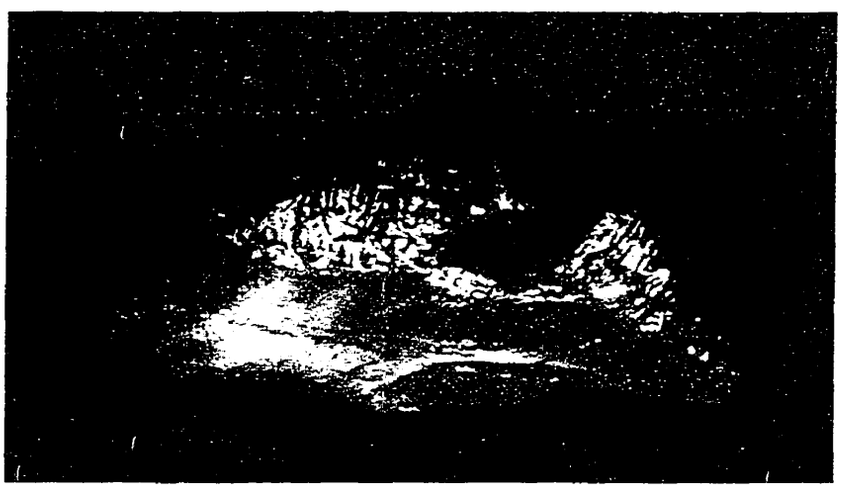


Fig. 3.1b Cross-section of the foot seen in fig. 3.1a. Small ulcer and fistula down to the dermis are visible.

Fig. 3.2 Severe hyperkeratosis on the hind feet.

Fig. 3. 3 Ulcer on main pad and swollen toe with ulceration around the nail bed.

Fig. 3.4 Severe exudation and necrosis of the underside of a front foot from a mink.

Fig. 3.5 Crusting above the nose and around the eyes in a mink kit. The kit also had lesions on the front feet.

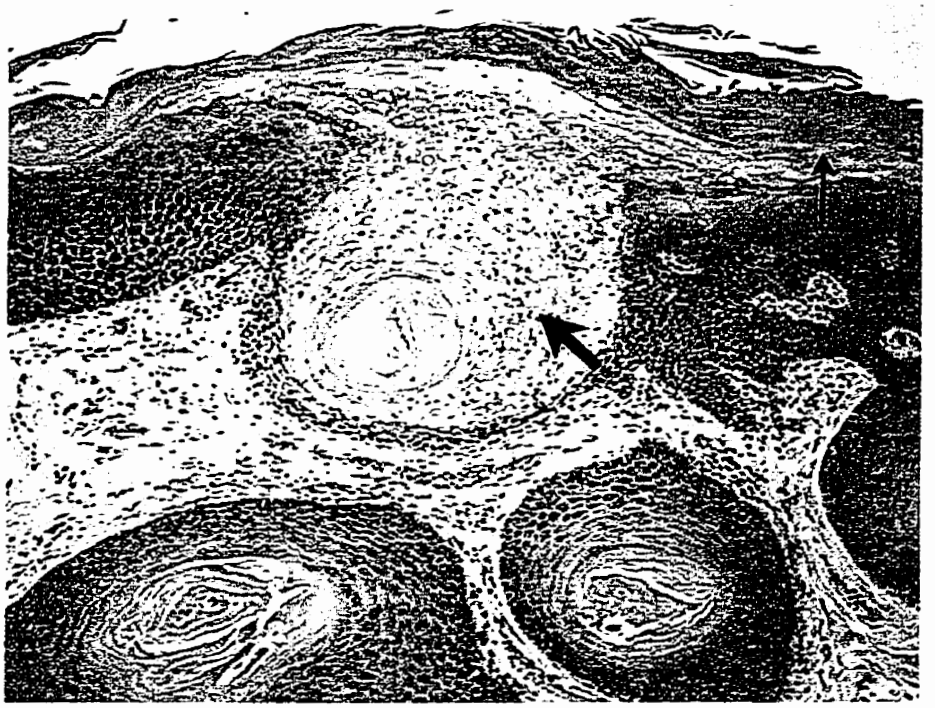


Fig. 3.6 Skin from a mink with hyperkeratosis of the metatarsal area. Note vacuolar degeneration of epitheliumof a hair follicle (thick arrow), hyperkeratosis (thin arrow) and mild lymphocytic subepidermal infiltration. H&E stain.

Fig. 3.7 Perifollicular lymphocytic infiltration (arrows) in skin section from mink foot with mild hyperkeratosis in metatarsal area. H&E stain.

Fig. 3.8 Skin section from mink foot with mild ulceration of the plantar surface. Note pigmentary incontinence, jumbled basal layer and mixed subepidermal inflammation with neutrophils dominating. H&E stain.

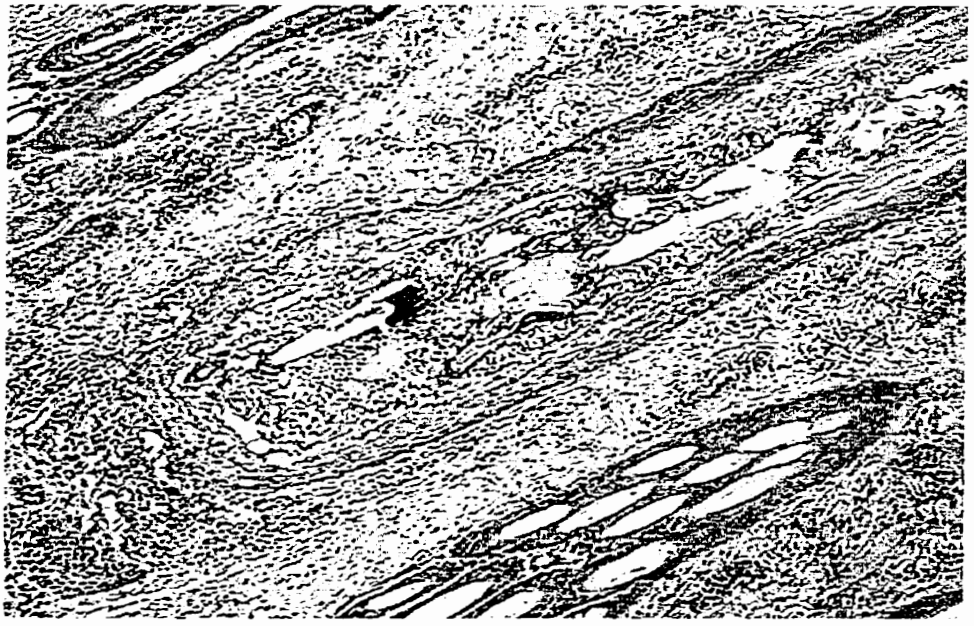


Fig. 3.9 Furunculosis in skin section from a mink foot with severe ulcers and hyperkeratosis. H&E stain.

CHAPTER 4

PODODERMATITIS IN FARMED MINK IN CANADA: POSSIBLE ETIOLOGIC AGENTS

4.0 Introduction

An apparently new skin disease affecting the feet of farmed mink (*Mustela vison*) was noticed in eastern and central Canada in the spring of 1996. The lesions most often observed were ulcerations, crusts and marked hyperkeratosis on the plantar and volar aspects of the metatarsal and metacarpal regions of the feet. Ulcers and crusts were also frequently seen around nail beds and in junctional areas where the pads join the furred skin. The earliest lesions found when inspecting a large number of affected live mink (n=130) and dead mink at pelting time (n=1471) were alopecia, swollen pads, mild hyperkeratosis and thick skin folds around toes (Bröjer, 2000, Ch. 3). In addition to the foot lesions, exudate, small ulcers and crusting were noted around the nose, around the eyes and on the facial skin up to the ears. Microscopically the lesions ranged from hyperkeratosis with peri- and mural folliculitis to ulcerations with furunculosis and marked dermal inflammation. The condition affected males more frequently than females. Morbidity was high on some farms whereas mortality was low. Mink farmers associated the emergence of the condition with the use of seal meat in the mink feed. There is epidemiologic evidence to support this view (Bröjer, 2000, Ch. 2). However, the cause of the disease is as yet unknown.

Foot lesions in mink have previously been described in association with hereditary tyrosinemia (Christensen et al., 1979), urolithiasis (Tomlinson et al., 1982), zinc-deficiency (Onderka, 1996) and distemper (Budd et al., 1966). These diseases were

considered in the process of attempting to determine the etiology behind the mink pododermatitis. The first three conditions were ruled out based on the macroscopic and histologic findings in the studied cases. Hereditary tyrosinemia was ruled out since typical ocular and renal lesions were absent. Furthermore, tyrosinemia has only been described in black mink (Sanford, 1988; Christensen et al., 1979), whereas the pododermatitis was seen in all color phases present on the farms (standard dark, mahogany, blue iris, demi buff, pastel) with no familial pattern. None of the mink had urolithiasis or any signs of uremia.

Zinc-deficiency was unlikely due to the epidemiologic pattern and localized nature of the lesions. Hyperkeratosis, crusts, scales, erythema, and exudation of other pressure points (elbows, hocks) as well as generalized seborrhea sicca, which are associated with zinc-deficiency in dogs (van den Broek and Thoday, 1986; Ohlen and Scott, 1986), were not observed in the mink with pododermatitis.

All mink were vaccinated against canine distemper (CDV). However, an atypical or new strain of distemper could not be completely ruled out based on macroscopic and histologic findings. Furthermore, many of the mink had been fed seal meat from harp seals (*Phoca groenlandica*) from eastern Canada, where phocine distemper has been documented (Daoust et al., 1993). Phocine distemper (PDV) has the potential to infect mink (Blixenkrone-Møller et al., 1989). Marked hyperkeratosis of the feet is a common lesion in distemper cases and, although typical inclusion bodies were not seen, some mink had interstitial reactions in the lung with prominent macrophage infiltration, which can be seen in distemper cases (Pinkerton, 1940, in Pearson and Gorham, 1987).

In dogs and cats, pododermatitis has been associated with infectious diseases (bacteria, dermatophytes, intermediate mycoses, parasites), allergic diseases (inhalant and

contact allergies), autoimmune diseases, endocrine diseases, environmental diseases (irritant contact dermatitis, trauma, plant awns) thallium toxicity, psychogenic dermatoses, and other hyperkeratotic and nodular diseases (zinc-responsive dermatosis, digital hyperkeratosis, plasma cell pododermatitis, neoplasia) (White, 1989).

The microscopic lesions in the mink feet were most consistent with a primary infectious disease or an environmental disease with secondary bacterial infections. There was no epidemiological evidence to support a single environmental agent as the cause of similar signs and lesions on different farms (Bröjer, 2000, Ch. 2). Histologic evaluation with haematoxylin and eosin and special stains (Periodic Acid Schiff, Whartin-Starry, Gram, and Ziehl-Neelsen) did not reveal the presence of fungi, parasites or spirochetes. Coccoid and rod-shaped bacteria were, however, present in many skin sections.

Based on the microscopic lesions, a viral etiology could not be ruled out. Some skin sections contained structures that resembled inclusion bodies in the cells of the stratum spongiosum in the epidermis and hair follicles. In some cases there was perifolliculitis without an obvious cause and several animals had interstitial reactions with large numbers of macrophages in lung sections. Except for distemper (caused by morbillivirus), dermatoses that are caused by viruses are rare in cats and dogs (Muller et al., 1989) and have, to the best of our knowledge, not been reported in mink. Poxvirus (Bennett, 1985), feline herpesvirus (Flecknell et al., 1979), and feline calicivirus (Cooper and Sabine, 1972) have been reported to cause dermatoses that involve the feet in cats. Caliciviruses of marine origin (San Miguel Sea Lion Virus-SMSV) can infect farmed mink experimentally fed infected seal meat or injected with SMSV (Wilder and Dardiri, 1978). Bacteriology; immunohistochemistry to identify distemper virus; calicivirus serology; and virus isolation were selected as tools to attempt to find a cause or causes of

pododermatitis in mink. It was known that some of the farms with pododermatitis also had Aleutian disease on their farm. In order to determine if there was an association between the foot lesions and Aleutian disease, selected serum samples were also analyzed for the presence of antibodies against Aleutian disease.

4.1 Materials and Methods

4.1.1 Bacteriology

Swabs for bacteriologic culture (Culturette™, Becton Dickinson Microbiology Systems, Becton Dickinson and Company, Sparks, Maryland, U.S.A.) were collected from a total of 51 feet with skin lesions, 18 skin lesions on the face (including eye and ear), and 22 spleens that were enlarged at the time of post-mortem examination. Swabs from eleven foot pads, one facial lesion and nine spleens were obtained from carcasses submitted by farmers for post-mortem evaluation. Swabs from nine feet, seven facial lesions and eight spleens were obtained from mink taken live from farms and killed with sodium pentobarbital in proplyene glycol (Euthanyl forte, MTC Pharamaceuticals, Cambridge Ontario, N3C 2W4) immediately before necropsy (University of Guelph Animal Utilization Protocol, 98R040). Swabs from 18 feet, five facial lesions and four spleens were taken from carcasses/feet that were collected at pelting time. Swabs from lesions on 13 feet and on five faces were obtained from live animals on farms.

The swabs were inoculated on typticase soy agar (Difco, Detroit, MI, USA) with 5% sheep blood and MacConkey (Difco) plates (Media Services, Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada) and incubated aerobically at 37°C for 48 hours. Plates were inspected after 24 and 48 hours. The amount of growth of different colony types was recorded as 4+, 3+,

2+, 1+ or zero. Bacteria with growth of 1+ were considered probable contaminants and were not included in the results. The different colony types were then sub-cultured on blood agar, re-inspected after 24 hours and Gram-stained. Presence and type of hemolysis was recorded. To differentiate staphylococci from streptococci, all gram-positive isolates were tested with H_2O_2 for catalase activity. All catalase-positive and hemolytic isolates were tested for coagulase activity (using rabbit serum on a glass slide and in a small test tube) and for acid production from fermentation of mannitol and maltose. Hemolytic staphylococci that were coagulase-negative on the slide and coagulase-positive in the tube were designated as *Staphylococcus aureus* if they were mannitol and maltose positive and as *Staphylococcus intermedius* if they were mannitol negative or weak positive and maltose negative or delayed positive. When the sugar fermentation tests were unclear, isolates (n=17) were submitted to the Animal Health Laboratory, Bacteriology Department, University of Guelph, for confirmation. Streptococci were only classified as hemolytic or non-hemolytic.

4.1.2 Immunohistochemistry- Distemper

In order to rule out canine distemper virus (CDV) and phocine distemper virus (PDV) as contributing factors to the disease, formalin-fixed, paraffin-embedded skin, lung, and urinary bladder sections were sent to Dr. Deborah Haines, Immunohistochemical Services, Diagnostic Immunology Laboratory, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada for immunohistochemical analysis. Twelve skin sections, eight lung sections and two bladders from a total of 14 mink cases were submitted. The skin sections selected had marked hyperkeratosis and small eosinophilic, inclusion-like material in the cytoplasm of

stratum spinosum cells. Lung sections that were selected had interstitial inflammatory reactions. The bladders had several vacuolated epithelial cells in the mucosa. In addition, skin and lung sections from a mink with clinical signs of distemper and histologic lesions compatible with distemper (eosinophilic inclusion bodies in skin and tracheal epithelium as well as interstitial pneumonia) were sent as a positive control.

An avidin-biotin complex immunoperoxidase histochemical stain was used to screen for the presence of morbillivirus-antigens in the sections (Haines and Chelak 1991). All sections were stained with 1/2000 and 1/4000 dilutions of a rabbit polyclonal antiserum against measles virus nucleoprotein and a negative control in which primary antiserum was omitted from the staining sequence. The rabbit polyclonal antiserum has previously shown to be widely cross-reactive to CDV, PDV and distemper virus of dolphins (Haines and Clark, 1991; Haines, 1998). The sections also were stained in duplicate with a monoclonal antibody to CDV, both times first with a horse anti-monoclonal antibody secondary and then with a biotinylated Protein A secondary (obtained from Dr. L. Elgren ADRI at Nepean, Ontario, Canada). Negative controls with omission of primary antisera were also included in the monoclonal CDV tests.

4.1.3 Serology

Calicivirus

Forty-two serum samples that had been collected from affected mink during pelting time (Bröjer et al., 2000, Ch. 2) were selected for calicivirus antibody analysis. A total of 24 serum samples were selected from six affected farms that were sampled in the winter of 1997. Seventeen of the samples were from mink with footpad lesions and seven were from clinically normal mink. Nine serum samples from mink on three non-affected (control) farms sampled during the same time period and nine serum samples from three

mink with lesions on affected farms sampled in the spring of 1998 also were analyzed. The samples were from 27 dark or mahogany, eight iris or sapphire and seven pastel or demi colored mink. Mink with different degrees of dermal inflammation were included.

Serum samples were sent to Dr. Alvin Smith, Calicivirus Laboratory, College of Veterinary Medicine, Oregon State University, U.S.A., where they were analyzed for the presence of antibodies against calicivirus using an ELISA technique. The ELISA was carried out in microtiter plates with 96 wells (ICN Catalog # 76-381-04. ICN Pharmaceuticals, Inc. Costa Mesa, California, USA). Fifty microliters of antigen (pool of San Miguel Sea Lion Virus, SMSV, numbers 5, 13 and 17) were incubated for two hours at 37°C on a shaker. The plates were washed twice with 370 ml Tris Buffered Saline (TBS: 0.05 M Tris, 0.15 M NaCl, pH = 7.4). The plates were then blocked three times with Pierce "Super Block" (Pierce Super Block ™ Blocking buffer in TBS. Pierce catalog # 37535. Pierce Chemical Co. Rockford, Illinois, USA) and stored at 4°C overnight. Test serum was diluted to 1:100 with TBST (TBS with 0.05% Tween 20) containing 0.25% bovine serum albumin (BSA). One hundred microliters of diluted sera were added and incubated two hours at 37°C on a shaker. After washing six times with 370 ml TBST, 100 ml secondary antibody (alkaline phosphatase labeled Protein A, Sigma P9650, Sigma-Aldrich, St. Louis, Missouri, USA, diluted in TBST containing 0.25% BSA) were added and incubated two hours at 37°C on a shaker. After washing six times with 370 ml TBST and six times with 370 ml TBS, 100 ml substrate was added and incubated three hours at 37°C on a shaker. Optical densities (OD) were read at 650 nm using a Microtiter Plate Reader (Titertek Instuments, inc.). A serum was considered positive if its corrected optical density (Od vs antigen - OD vs control) was greater or equal to 0.200 and serum vs. antigen optical density was greater than or equal to two times the serum vs. no antigen

optical density. The cut-off points were based on previous SMSV serologic studies (Smith, 1999).

Aleutian Disease

Serum samples from the same 42 mink that were sent for calicivirus serology, were analyzed at the Animal Health Laboratory (AHL), University of Guelph, Guelph, Ontario, Canada for the presence of antibodies against Aleutian disease virus (ADV) by counter immunoelectrophoresis. An additional 17 mink sera, collected prior to euthanising mink for post-mortem evaluation were also tested for ADV antibodies.

4.1.4 Virology

Samples were sent to Dr. Alvin Smith at the Calicivirus Laboratory, College of Veterinary Medicine, Oregon State University, Oregon, U.S.A. for virus culture. Samples were sent from nine mink and included eight rectal swabs, six pharyngeal swabs and three swabs from skin lesions that had all been frozen at -70°C in virus-transport medium (appendix 4.1). Swab samples were vortexed and centrifuged for 10 minutes at 400x g. Of each resulting supernatant, 200µl was inoculated onto 24-hour-old cell monolayers in tissue tubes. The tubes were incubated for one hour at room temperature, the inoculum was removed, and 1.5 ml of growth medium (MEM consisting of 1% calf serum plus antibiotics) was added to each. Tubes were then incubated at 37°C on a roller drum rotating at 1/3 rpm and observed daily by light microscopy for viral cytopathology. After seven days, cultures were freeze-thawed and passed to new roller tubes, following the procedure above. Samples were passed at least three times on two cell lines: VERO African Green Monkey Kidney (VMK) and Porcine Kidney (PK-15). Cell cultures exhibiting viral cytopathology normally are processed for negative stain transmission electron microscopy and viral isolates are serotyped using a standard serum neutralization

test (100 $TCID_{50}$ virus against 20 antibody units of typing sera) but since no cytopathologic effect was seen, these last steps were not performed.

Some tissue samples were cultured by the Animal Health Laboratory, University of Guelph. Samples were collected from four necropsied mink (lung, oral swab, skin lesion swab from each) and from four live mink (three skin lesion swabs, four rectal swabs and one eye swab) and were frozen at -70°C in virus transport medium (appendix 4.1). Cultures of these samples were passaged seven times (each passage was one week) on VERO, canine A72, Crandell Feline Kidney (CrFK) and PK15 cell lines. The samples from the live mink were passaged five times on mink lung, VERO and CrFK cell lines. Parvovirus hemagglutination was performed on supernatant medium from all cell lines using monkey red blood cells.

4.2 Results

4.2.1 Bacteriology

Bacteria were isolated from 50 (98%) of the feet swabs (Table 4.1). *Staphylococcus intermedius* was isolated in pure culture from 15 (29.4%) cases and in mixed culture in eight (15.7%) cases. In the mixed cultures the most common other isolates were non-hemolytic streptococci and *E. coli*. *Staphylococcus aureus* was isolated in pure culture in two (3.9 %) case and in mixed culture in 3 (5.9%) cases. *Staphylococcus epidermidis* was isolated in pure culture in two (4%) cases and in mixed cultures in six (11.8%) cases (including one with *S. aureus*, which is included in the *S. aureus* statistic above as well). Beta-hemolytic streptococci were isolated in pure culture in one (2%) case. In 11 cases there was overgrowth of *Proteus* spp.

As in cultures from feet, S. *intermedius* was the organism most often isolated in pure or mixed culture in samples from the head region. *S. intermedius* was isolated in pure culture from five (26.3%) samples and in mixed culture from four (21.1%) samples. Only one spleen (4.5%) showed any bacterial growth (hemolytic streptococci).

4.2.2 Immunohistochemistry

Using the polyclonal antiserum to measles virus nucleoprotein, there was some non-specific staining of epithelium in some skin sections with hyperkeratotic lesions. The known mink distemper case was clearly positive. When the sections were stained with the monoclonal antibody to CDV, there was no convincing specific staining except in the mink distemper case and a canine brain positive control. The tested sections thus were considered negative for canine and phocine distemper viruses.

4.2.3 Serology

Calicivirus

One mink serum sample out of 42 analyzed was positive (OD vs. Antigen = 0.629, OD vs. serum control = 0.013, corrected OD = 0.616) when tested for antibodies against a combined sample of SMSV 5, 13 and 17. The positive serum came from a pastel mink with deep ulcerations on all four feet. It was sampled in March 1998 during the spring pelting time.

Aleutian disease

Seven (12%) out of 59 sera had antibodies against Aleutian disease virus. Five of those came from one farm and each of the other two came from a different farm. Three of the positive sera were from animals with histologic changes compatible with Aleutian disease (Bröjer et al., 2000 chapter 3). Serologic results are not available for the

remaining three mink with histologic changes compatible with AD since no serum was available from these animals.

4.2.4 Virology

Virus isolation attempts were negative at both the AHL and the Oregon State University laboratory.

4.3 Discussion

Bacterial growth was obtained from most of the cultured foot and facial lesions and the predominant bacteria was *Staphylococcus intermedius*. Staphylococci and streptococci are often associated with skin infections in individual mink but seldom cause a herd problem (Onderka, 1996). Staphylococci are considered normal inhabitants of the skin and mucocutaneous junctions of animals (Jonsson and Wadstöm, 1993). The normal skin flora is divided into a resident and a transient population. The resident population includes those microorganisms that form stable colonies on the skin surface and proliferate there. The transient population includes those microorganisms that are deposited on the skin and are able to persist for a short period of time. (Lloyd, 1993). In dogs, coagulase-positive staphylococci such as *S. intermedius* are considered part of either the resident skin flora (Yager and Scott, 1993) or part of the nomad flora (Lloyd, 1993). Nomad organisms fall between residents and transients and are defined as organisms that are readily able to take advantage of changes in the skin surface microenvironment and thus frequently become established and proliferate at the skin surface and in deeper tissues. *S. intermedius* has previously been isolated from the rostral nares of apparently healthy mink (Hajek, 1976) suggesting that this organism probably is a normal inhabitant in mink just as in dogs.

Superficial bacterial folliculitis in dogs is most often associated with *S. intermedius* (Muller et al., 1989 a). *S. intermedius* can be pathogenic to mink, causing clinical mastitis and vaginitis in adult females and dermal adenitis in neonates (Hunter and Prescott, 1991). Coagulase-positive staphylococcal species also are known to cause dermatitis (Crandell et al., 1971), mastitis (Budd et al., 1966; Trautwein and Helmboldt, 1966; Ryan et al., 1979), urolithiasis (Sompolinsky, 1950; Tomlinson, 1982), and enterotoxicosis (Juokslahti et al., 1980) in mink.

It is possible that *S. intermedius* plays a role in the pathogenesis of pododermatitis in mink. However, S. *intermedius* was not isolated from all of the cultured lesions. Furthermore, staphylococcal pyoderma in other species, such as the dog, often is secondary to other predisposing factors, including continued moisture, alterations in keratinization, frictional damage, physical irritation due to external parasites and their secretions, self-induced damage, accumulated dirt, excess sweating, and direct trauma (Jonsson and Wadstöm, 1993; Yager and Scott, 1993).

The hyperkeratosis observed on footpads and on the plantar and volar surfaces of the metatarsal and metacarpal regions (Bröjer, 2000, ch. 3) might represent a primary lesion that could contribute to bacterial infection. The hyperkeratosis could, however, also be a reaction to a chronic infection with another primary etiology.

Distemper was eliminated as a primary cause of hyperkeratosis since the immunohistochemical analyses were negative. The polyclonal measles antiserum produced some staining of epithelial cells but no staining was observed with the monoclonal canine distemper antiserum. It is possible that the latter did not pick up phocine distemper or a mutated distemper antigen. However, this scenario is unlikely since the staining was non-specific using the polyclonal antiserum (no cellular inclusions

or specific cell-types were stained) and the different distemper viruses have previously been shown to crossreact serologically (Haines and Clark, 1991). Furthermore, the mink did not show any other evidence of clinical disease resembling distemper, even though mink infected with phocine distemper virus have shown similar clinical signs as when infected with canine distemper virus (Blixenkrone-Møller et al., 1989).

Frictional or traumatic damage to the feet could be a contributing factor to the pododermatitis, since the mink are housed in wire cages. Ulcerative pododermatitis, also referred to as "sore hocks", sometimes is seen in rabbits that live in wire cages (Flatt et al. 1974; Marcato and Rosmini, 1986). The lesions resemble those seen in mink and include hyperkeratosis and circumscribed ulcerated lesions covered by a reddish or blackish dry crust located on the plantar surface of the metatarsal region and, occasionally, in the metacarpal-phalangeal region. Predisposing factors are heavy body weights, use of wire-floored cages, and accumulation of urine and feces in the cages. The lesions are often complicated by secondary invasion of bacteria, especially *Staphylococcus aureus* and *Fusobacterium necrophorum.* Similar lesions have been described in guinea pigs kept in cages with wire floors (Sirois, 1989).

Though the mink lesions resemble those seen in rabbits and guinea pigs, it is difficult to explain why a large number of farmers started seeing the foot problems in many of their mink at about the same time (1996). The mink had been housed in the same type of cages for many years without foot problems and no other changes had been made in housing or cleaning routines when the lesions began appearing (Bröjer, 2000, ch. 2). Nonetheless, it is possible that the wire caging exacerbates skin abrasions caused by another primary factor. The fact that mink often stand on their hind feet, thus applying more pressure to them than on the front feet, may explain the higher prevalence of lesions

in the hind feet. The higher incidence in males during the breeding season could reflect the increased anticipatory pacing of males prior to breeding.

Viruses such as calicivirus, herpesvirus and poxvirus, that cause blisters or alterations in the epidermis that could serve as a port of entry for bacteria, were not isolated. Virus isolation was attempted mostly on material that had been frozen (-70°C) which could have affected virus viability. Since most of the lesions observed and sampled were chronic, it is possible that the virus was no longer present at the time of sampling.

One mink had a high antibody titer against San Miguel Sea Lion Virus (SMSV-calicivirus) but no conclusions can be drawn from one positive animal. The duration and magnitude of antibody titers against SMSV depend on route of infection, species and individual. Experimental intradermal and intranasal infection of harp seals with SMSV-2 resulted in low virus neutralizing activity in serum on day 3 through 7 but when tested again at days 43 and 44, neutralizing antibody levels in individual seals had decreased to less than 1 log (Gelberg et al., 1982). Mink that were experimentally fed SMSV-5 in different concentrations had antibody titers of zero, 1:4 and 1:64 fourteen days post-infection (Wilder and Dardiri, 1978). Twenty-one days post-infection some of the titers had gone up while others had gone down to zero. It is, therefore, possible that more mink had positive antibody titers but that blood samples were not taken at appropriate times or that the mink developed very low antibody levels. More positive mink would nonetheless have been expected if antibodies had been present since the analyzed samples were chosen to represent mink with different stages of lesions as well as mink with inapparent lesions. Another complicating factor was that Protein-A rather than a specific anti-mink

serum antibody was used in the test, which could contribute to lowering the sensitivity of the test. In that case, some of the negative results could be false-negatives.

Macroscopic and microscopic evaluation of pododermatitis lesions, as well as epidemiologic data, are consistent with an infectious etiology. The etiologic agent seems to cause a small lesion that can worsen as a result of mink pacing the wire cages, allowing secondary bacterial invasion. Bacteria were isolated from most of the cultured lesions. Gram-positive cocci and a few gram negative bacteria, also were observed microscopically in many ulcerated skin sections, but no other infectious agents were identified. *S. intermedius* was isolated in pure or mixed culture in approximately half of the cases, but very few of the earliest lesions were cultured since the skin was usually intact. Early lesions had more peri- and mural folliculitis than luminal folliculitis and bacteria were not found microscopically in those lesions. Because of the mixed nature of the cultures and the fact that *S. intermedius* is a commensal, bacteria were not considered to be the primary pathogens of mink pododermatitis

Known or unknown viruses were potential primary pathogens. No viruses were identified in cell culture or with electron microscopy and only one mink was serologically positive to SMSV. Canine and phocine distemper were ruled out based on immunohistochemistry. A viral etiology can not, however, be ruled out completely since very few early lesions were found and analyzed. Potential viruses are probably difficult to detect in older lesions with secondary bacterial infections.

To further explore mink pododermatitis it is, therefore, essential to find more early lesions. This could perhaps be achieved by daily monitoring of a group of young (4-5 month old) mink on an infected farm. Once early lesions are found, both bacterial and viral culture should be attempted. For comparison, samples for bacterial culture should

also be taken from normal mink feet. Serum should also be tested for SMSV antibodies with specific anti-mink serum antibody. Molecular methods such as staining with monoclonal antibodies could be used on representative lesions to screen for SMSV or other potential pathogens.

Table 4.1. Prevalence (%) of different bacterial species isolated in pure or mixed cultures from mink with skin lesions associated with pododermatitis.

Bacteria	Feet (n=51)	Face (n=19)	Spleens (n=22)
Staphylococcus intermedius	29.4	26.3	0
S. intermedius + Streptococci &/or *E. coli*	13.7	21.1	0
S. intermedius + *Staphylococcus epidermidis*	2.0	0	0
Staphylococcus aureus	3.9	5.3	0
S. aureus + Streptococci &/or *E. coli*	5.9	5.3	0
Staphylococcus epidermidis	3.9	5.3	0
S. epidermidis + Streptococci &/or *E. coli*	9.8	5.3	0
non-hemolytic Streptococci & *E. coli*	3.9	10.5	0
Hemolytic Streptococci	2.0	0	4.5
Hemolytic Streptococci + *E. coli*	2.0	0	0
Proteus overgrowth	21.5	15.6	0
Negative	2.0	5.3	95.5

CHAPTER 5

5.0 Summary and General Discussion

This study investigated the epidemiology, pathology and possible causes of an apparently "new" form of pododermatitis affecting farmed mink (*Mustela vison*) in central and eastern Canada first identified in 1996. Farmers saw mink with marked hyperkeratosis and ulcers primarily on the plantar and volar aspects of the feet. They noticed that males were more often affected and that they were unwilling to breed, presumably due to their sore feet. Many farmers associated the condition with the use of seal meat in the mink feed ration. Since seal meat is a relatively cheap source of high quality protein and since the pododermatitis had the potential to cause economic losses, the Canada Mink Breeders' Association (CMBA) proposed an investigation of the disease and its possible association with feeding seal meat. The study has not been able to determine the cause or causes of the disease but the results suggest there is an infectious component involved and that seal meat may have been involved in the initial outbreaks.

The epidemiologic study showed that there was a strong statistical association between feeding seal meat and the presence of pododermatitis on the farms. When evaluating the significance of this statistical association the limitations of this part of the study must also be considered. The main problem was its low power due to the low response rate to the questionnaire (34%). A telephone follow-up of the questionnaire could have increased the response rate. A pilot study asking a few farmers to fill out the questionnaire may have lead to re-wording of some of the questions in order to obtain more clear-cut answers. Despite the limitations of the survey, it confirmed that

pododermatitis was a problem among many mink on at least 22 farms and that seal meat could in some way be involved in the spread of the disease.

The link between seal meat and pododermatitis is strengthened by evidence of a similar foot condition observed in mink in Utah, USA in the early 1970's. The Utah mink had been fed meat from northern fur seals (*Callorhinus ursinus*) harvested on the west coast of Alaska, USA (Larsen, 1997). The condition disappeared some time after the use of seal meat was discontinued. It is possible that the seal meat in both cases contained an infectious agent that could be transmitted to mink. Although the sample size of different age, gender and color categories of inspected mink in the present study was small and varied, the epidemiological data further support the theory that there is an infectious agent involved. The owner of the only affected farm in which seal meat had not been used in the feed ration, reported that his mink acquired pododermatitis only after the introduction of animals from an affected farm. The prevalence of disease among juveniles at pelting time was high and kits from affected parents developed lesions shortly after weaning. However, on most farms, the majority of the affected juvenile animals had not been fed seal meat, which suggests that though seal meat may have been an initial contributing factor, it was not necessary for the propagation of the disease.

The fact that males were more often affected than females does not support an infectious etiology since both genders would be expected to be equally represented. However, if the initial insult was very small, the lesion could be inapparent and possibly heal quicker in females due to their lighter weights. The males' heavier weights could lead to increased friction between the feet and the cage's wire floor increasing the risk of ulceration. Ulcers could then act as a port of entry for secondary bacterial infections, thus worsening the lesions.

The macro- and microscopic findings also supported a pathogenesis involving an infectious agent. The earliest visible lesions included alopecia and thickening of the skin on the plantar and volar aspects of the feet as well as thickening of the interdigital skin resulting in folds that resembled thick-walled blisters without fluid. The alopecic areas were occasionally covered by a small amount of exudate. The number of observed mink with this type of lesion was limited but these types of lesions strongly suggest that the initial insult was not bacterial. The lesions progressed with formation of small fistulas, ulcers, crusts and hyperkeratosis. Histology revealed increasing degrees of epidermal and follicular hyperkeratosis, peri-follicular and follicular inflammation, ulceration and dermal inflammation. The lesions were generally non-specific but were consistent with an inflammatory reaction against an unknown agent followed by secondary bacterial infection.

Staphylococcus intermedius was the most common bacterial isolate from skin lesions. *Staphylococcus intermedius* is the primary bacterial pathogen of canine skin (Ihrke, 1987) and has been isolated from mink kits with dermal adenitis (Hunter and Prescott, 1991). However, other bacteria including *Staphylococcus aureus*, non-hemolytic staphylococci, *Streptococcus* sp., *E. coli* and *Proteus* sp. were also isolated from the mink feet and *S. intermedius* was not isolated from all cases. *Staphylococcus intermedius* is a skin commensal and usually does not cause disease unless there is a disruption of the cutaneous ecosystem or a failure of the host's defense response (Mason et al., 1996). It is therefore likely that there is an unknown predisposing factor leading to the bacterial infections.

The possibility of a primary viral infection was pursued since viruses are often difficult to find and can cause a variety of lesions. Canine and phocine distemper were

ruled out because typical clinical signs were absent and antigen was not detected with immunohistochemical staining. Virus isolation attempts were negative and no association was found between pododermatitis and Aleutian disease. Calicivirus serology against San Miguel sea lion virus (SMSV) type 5, 13 and 17, which crossreact with more than 30 SMSV serotypes (Smith, 1999), yielded one positive mink sample out of 26 tested with clinical signs. All mink without signs (seven from affected farms and nine from non-affected farms) tested negative. Although no conclusions can be drawn from this result alone, its importance increases in light of serologic test results from Canadian East coast harp seals (*Phoca groenlandica*) sampled in 1998. Seven out of 37 seal samples had antibodies against SMSV (Bröjer et al., unpublished), which strongly suggests that SMSV is present in the North American east coast seal population. SMSV has the potential to infect mink (Wilder and Dardiri, 1978) and can survive freezing (Sawyer et al, 1978). The virus could therefore potentially survive in frozen seal meat and can not be ruled out as a possible etiologic agent.

The seal samples were analyzed using both Protein-A and anti-harp seal immunoglobulin as secondary antibodies in an ELISA test. Analysis using protein-A yielded one out of 37 positive samples whereas use of anti-harp seal immunoglobulin yielded seven out of 37 positive samples. The mink samples were only analyzed using Protein-A. It may therefore be of value to re-run mink samples using a specific anti-mink immunoglobulin. Monoclonal antibodies conjugated to fluoresce in-situ with calicivirus infected cells are also available (Smith, 1999) and could be used to evaluate histologic sections from infected feet with early lesions.

Further studies of the pododermatitis affecting mink are necessary to fully understand the etiology, pathogenesis and progression of the lesions. At the end of 1998

several of the farmers whose mink were initially affected, perceived a marked decrease in the number of affected animals. A follow-up questionnaire, preferably with telephone contact, would be helpful to determine how widespread the condition is currently and whether farms that were initially affected still are affected. Daily monitoring of a group of animals on affected farms for a period of time could be helpful in finding early lesions. Early lesions are essential in order to obtain a better description of the course of the disease and to be able to utilize some of the more advanced technology available. Staining with monoclonal antibodies against caliciviruses, other immunohistochemical tests or perhaps PCR could be used to clarify the role of *Staphylococcus intermedius* and to look for other specific etiologic agents.

CHAPTER 6

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6.1 Appendix 2.1 Questionnaire about pododermatitis sent to mink farmers

Name
Address
Telephone

Animals
How many breeding females do you have?
How many breeding males do you have?
How many females of each color phase do you have?

Color	Number

Have you bought any new animals during the last year?
Yes- What month?
No

Housing
Are the mink housed in "standard" wire pens?
Yes
No-They are housed in_____

What kind of nesting box do you have?
Overhead nest box
Drop-in nest box
Wire nest box
Other_____

What kind of bedding material do you have?
Straw
Shavings/sawdust
Straw and shavings
Chopped excelsior
Shredded beet pulp
Other_____

Feed

What types of mink feed do you use?

Commercial ready mix
Mix own feed

Have you used seal meat or seal byproducts in the feed in the last year?

Yes
No

If you have used seal meat,

During which months of the year did you use it?
How did you use/feed the seal meat?

Raw
Cooked

What per cent of the diet did the seal meat comprise?
What was your source of seal meat?

Disease

Have you noticed any animals with lesions on their feet and/or legs resembling those described in the introduction?

Yes
No

If yes,

In what months did the lesions occur?

Please fill out the table on age and sex of affected animals:

	Number Affected	
	Adults	Kits
Females		
Males		
Total		

What color phases were affected?

Color	Number

Did you give the animals with this condition any medication?

Yes- What? and How was it administered? _____
No

Was the treatment successful?
Yes
No

Do you use routine or periodic antibiotic "flushing" programs?
Yes- What? and How administered?
No

How many animals were found dead from this condition?

How many animals were euthanized (killed) due to this condition?

Were any of these animals autopsied?
Yes- Diagnosis_____
No

Many diseases of mink can cause similar foot lesions. These include Aleutian disease, distemper, hereditary tyrosinemia and kidney infections. We therefore wonder if your mink have had any of these diseases?
No
Yes, Distemper
Yes, Aleutian disease
Yes, Hereditary tyrosinemia
Yes, kidney infection

What do you vaccinate your mink against?
Botulism
Distemper
Pseudomonas aeruginosa
Mink virus enteritis

6.2 Appendix 2.2 Summary of survey results

	Total	Seal + Dis§	Seal + No Dis	No seal + Dis	No seal + No Dis
ANIMALS					
Total adult female mink- all farms	147,180	45,090	34,090	650	67,350
Total adult female mink- farms reporting number affected†	140,480	38,390	34,090	650	67,350
Total adult male mink- all farms	24,850	8,088	5,993	150	10,619
Total adult male mink- farms reporting number affected	24,110	7,348	5,993	150	10,619
Number affected adult female	99	96	0	3	0
Number affected adult male	470	467	0	3	0
Adult females eating seal meat	99/73130 = 0.14%	99/39040 = 0.25% (0-3.23%)			
Adult males eating seal	470/13491 = 3.48%	470/7498 =6.26% (0-17.5%)			
HOUSING					
Standard wire pen	72/73 (99%)	21/21 (100%)	18/19 (94.7%)	1/1 (100%)	32/32 (100%)
Overhead nest	15/73 (20.5%)	4/21 (19%)	4/19 (21.1%)	0/1 (0%)	7/32 (21.9%)
Drop-in nest	56/73 (76.7%)	17/21 (80.1%)	15/19 (/8.9%)	1/1 (100%)	23/32 71.9%
Wire nest	5/73 (6.8%)	1/21 (4.8%)	1/19 (5.3%)	0/1 (0%)	3/32 (9.4%)
Other housing	20/73 (27.4%)	5/21 (23.8%)	6/19 (3.7%)	0/1 (0%)	9/32 (28.1%)
BEDDING					
Straw	23/73 (31.5%)	7/21 (33.3%)	3/19 (15.8%)	1/1 (100%)	12/32 (37.5%)
Shavings/ sawdust	49/73 (67.1%)	16/21 (76.2%)	12/19 (63.2%)	1/1 (100%)	20/32 (62.5%)
Straw/shavings	17/73 (23.2%)	5/21 (23.8%)	6/19 (3.2%)	0/1 (0%)	6/32 (18.8%)
Excelsior	2/73 (2.7%)	0/21 (0%)	1/19 (5.3%)	0/1 (0%)	1/32 (3.1%)
Beet pulp	3/73 (4.1%)	2/21 (9.5%)	1/19 (5.3%)	0/1 (0%)	0/32 (0%)

Summary of survey results (continued)

	Total	Seal + Dis§	Seal + No Dis	No seal + Dis	No seal + No Dis‡
FEED					
Ready food	18/73 (24.6%)	3/21 (14.3%)	5/19 (26.3%)	0/1 (0%)	10/32 (31.3%)
Own food mix	55/73 (75.4%)	18/21 (85.7%)	14/19 (73.7%)	1/1 (100%)	22/32 (68.7%)
Raw seal meat	40/40 (100%)	21/21 (100%)	19/19 (100%)	----------	----------
Mean % seal in feed	10.65%	11.06%	10.25%	----------	----------
ANTIBIOTIC FLUSH	24/50 (48%)	9/21 (42.9%)	7/14 (50%)	1/1 (100%)	7/14 (50%)
DISEASES					
Distemper	2/68 (2.9%)	0/21 (0%)	1/19 (5.3%)	0/1 (0%)	1/27 (3.7%)
Aleutian Disease	15/68 (22.1)	4/21 (19%)	5/19 (26.3%)	1/1 (100%)	5/27 (18.5%)
Tyrosinemia	9/68 (13.2%)	4/21 (19%)	2/19 (10.5%)	0/1 (0%)	3/27 (11.1%)
Kidney disease	4/68 (5.9%)	2/21 (9.5%)	0/19 (0%)	0/1 (0%)	2/27 (7.4%)
VACCINES					
Vacc. botulism	65/67 (97%)	21/21 (100%)	19/19 (100%)	1/1 (100%)	25/26 (96.2%)
Vacc. distemper	67/67 (100%)	21/21 (100%)	19/19 (100%)	1/1 (100%)	26/26 (100%)
Vacc. pseudomonas	27/67 (40.3%)	10/21 (47.6%)	8/19 (42.1%)	1/1 (100%)	8/26 (30.8%)
Vacc. Mink virus enteritis	66/67 (98.5%)	21/21 (100%)	19/19 (100%)	1/1 (100%)	25/26 (96.2%)

§ Seal = Farms that had fed seal meat. Dis = farms affected by pododermatitis.

† Three farms did not specify the number of animals with pododermatitis. These farms were excluded in the prevalence analysis.

‡ All of the farms in this category did not responded to the questions about diseases and vaccine routines.

6.3 Appendix 4.1 Virus Transport Medium

For 500ml:

10X Hanks with Phenol Red	50 ml
Lactalbumin Hydrolysate (0.5%)	2.5 gm
Hepes (25mM)	3.54 gm
Double distilled water	240 ml

Mix and set pH at 7.2

Autoclave at 116 C for 15 minutes on slow exhaust. When cool add:
62,500 units/500ml Penicillin
62,500 micrograms/500ml Streptomycin
25,000 units/500ml Mycostatin