# EPIDEMIOLOGY AND PATHOLOGY OF NEOPLASIA IN THE CAPTIVE POPULATION OF THE BLACK-FOOTED FERRET (*Mustela nigripes*)

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## ABSTRACT

EPIDEMIOLOGY AND PATHOLOGY OF NEOPLASIA IN THE CAPTIVE POPULATION OF BLACK-FOOTED FERRET (*Mustela nigripes*)



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This thesis presents the findings of a retrospective study on neoplasia in the captive population of black-footed ferrets. Of the 227 adult ferrets (> 1-yr-old) that died since the beginning of the captive propagation programme, 184 could be included in this study based on the availability of information and material. Clinical files, postmortem reports, and archived tissues were reviewed for each case. A total of 185 neoplasms, of 28 different phenotypes, was diagnosed in 102 of the 184 adult ferrets examined, for a prevalence at death of 55.4%. The crude annual incidence and the life-span adjusted incidence of neoplasia in this population for the four last years of the study were respectively estimated to be 4458 and 446 cases per 100,000 animals. Multiple neoplasia was common, 51 ferrets were affected by more than one tumour. Renal tubular neoplasms, biliary cystadenoma/carcinoma, and tumours of the apocrine gland were the most commonly encountered neoplasms. Although etiology and risk factors for renal tubular neoplasms could not be determined, a possible familial predisposition for this malignancy was hypothesized on the basis of the exceptionally high occurrence, the

common multifocal and bilateral manifestation, the limited genetic diversity of this population, and the absence of similar cases in black-footed ferrets from South Dakota. The histology of the biliary tumours strongly suggested that these neoplasms originate in intrahepatic biliary cysts. These cysts, which were very common in this population (prevalence at death of 66%), are believed to be associated either with a congenital or an acquired defect in the mechanisms controlling the differentiation of biliary progenitor cells. Due to uneven gender distribution, neoplasms of the apocrine glands are believed to be under hormonal influence. Neoplasia is an important cause of mortality of adult ferrets, but the impact on the captive propagation of this species is probably limited, since tumours are encountered almost exclusively in post-reproductive ferrets. The effect on the wild population would also probably be insignificant, since ferrets released into their natural habitat rarely reach the age when these neoplasms occur.

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# 1 INTRODUCTION

The saga of the decline of the black-footed ferret (*Mustela nigripes*) and of its salvage *in extremis* from extinction is an excellent example of problematic interactions between people and wildlife. Once thriving throughout the great plains of North America, this highly specialised predator was victim of its intimate association with its prey, prairie dogs (*Cynomys* spp.). Prairie dogs were believed to compete with introduced livestock, and therefore were widely poisoned by ranchers. This extensive subsidized eradication campaign caused almost complete collapse of the prairie dog ecosystem. This was associated with an inevitable decline of species dependant on this habitat, such as the black-footed ferret (DeBlieu, 1991). With the death in 1979 of the last captive black-footed ferret, the species was believed to be extinct; however, an isolated population was discovered near Meeteetse, Wyoming in 1981 (DeBlieu, 1991).

An outbreak of distemper in 1985 greatly jeopardized the survival of this last population, and forced the capture of the last 18 known free-ranging black-footed ferrets in 1986 and 1987 (Thorne and Williams, 1988). This was the beginning of one of the most successful captive propagation programs in the history of conservation of endangered species. This program, initiated and first managed by the Wyoming Game and Fish Department, today is under the supervision of the United States Fish and Wildlife Service, and involves one central breeding facility and six satellite breeding centres.

The current captive population of black-footed ferrets is issued from only seven founders with descendants represented in the population, and therefore has a low degree

of genetic variation (O'Brien et al., 1989). Loss of genetic heterogeneity in small populations may be associated with low survival rates, the result of unmasking of deleterious recessive genes, loss of reproductive fitness, or loss of variation in immune function (Munson, 1993). In order to detect potentially deleterious traits in a genetically homogeneous population, it is essential to monitor causes of morbidity and mortality. Several possibly genetic defects, such as short tail, misshapen canines and renal aplasia have been detected in captive-born black-footed ferrets (Godbey and Biggins, 1994).

Several black-footed ferrets captured from the South Dakota population developed adnexal tumours, and it was speculated that a genetic predisposition associated with inbreeding might have played a role in the development of these neoplasms (Carpenter et al., 1981). However, this hypothesis was based on only five animals. Neoplasms are also very commonly diagnosed in captive adult ferrets issued from the Meeteetse population. This high occurrence of neoplasia is intriguing, and suggests a potential genetic predisposition, or exposure to carcinogenic factors. The possible link between these tumours and host or environmental factors has never been explored. In addition, the impact of these neoplastic conditions on the captive propagation program has not been evaluated.

The purpose of this study is to better characterize the neoplastic diseases present in the captive population of black-footed ferrets, in order to identify potential predisposing factors, and to assess the significance of these conditions for the management of the species.

# 2 LITERATURE REVIEW

In this literature review the biology of the black-footed ferret will be explored and the principal steps involved in the management of this endangered species will be summarized. The literature on neoplastic diseases in Mustelidae will also be reviewed. Finally, some factors that can be associated with a high incidence and clustering of neoplasia in a population will be examined.

## 2.1 Biology and conservation of the black-footed ferret

### 2.1.1 *Mustela nigripes*

The black-footed ferret, the only member of the subgenus *Putorius* native to the Americas, was first described in the scientific literature in 1851 by Audubon and Bachman (1851). This ferret, closely related to the Siberian polecat (*Mustela eversmanni*), and the Europan polecat (*Mustela putorius*), once inhabited short and mid-grass prairies of 12 American states and two Canadian provinces (Anderson et al., 1986). The range of this highly specialized predator, adapted for life in prairie dog burrows, closely matched the distribution of the various species of prairie dogs, which constituted a large proportion of its diet (Campbell II et al., 1987). Besides this strong predator-prey relationship, black-footed ferrets also rely on underground tunnels excavated by prairie dogs for shelter against predators and harsh climate.

### 2.1.2 Cause of decline of the species

Due to the belief that prairie dogs were competing with livestock for pasture, and to the danger to horses falling into burrows, poisoning of all five species of prairie dogs began by the late 1880s. The eradication program accelerated in 1915 when it began to be supervised and subsidized by federal agencies (Bishop and Cullbertson, 1976). By 1960, the historical distribution of prairie dogs had decreased from 40 million hectares to only 600 000 hectares (Marsh, 1984). The inevitable decline of the black-footed ferret, predicted in 1926 by Seton (1926), was signalled by the very small number of animals seen after 1946 (DeBlieu, 1991).

In 1964, the black-footed ferret was about to be added on the list of extinct species when a small isolated population was discovered in South Dakota. Despite the designation of the black-footed ferret as an endangered species in 1966, the crusade to consolidate this last known population was compromised by the lack of collaboration of local landowners, and by continuation of the prairie dog eradication program (Linder et al., 1972). This population slowly collapsed, and after 1974 no more free-ranging black-footed ferrets were seen in South Dakota (Hillman and Carpenter, 1983). After the death in 1979 of the last captive black-footed ferret at the U.S. Fish and Wildlife Service Research Center in Patuxent, Maryland, the species was believed for the second time to be extinct (Carpenter, 1985).

However, in September 1981 another population was discovered near Meeteetse, Wyoming, when a black-footed ferret was killed by a farm dog. Subsequent surveys indicated that the size of this isolated population was stable at around 100 animals, but in

1985 the extremely low number of black-footed ferrets seen suggested a possible crash of this last population (Forrest et al., 1988). In October 1985, an epidemic of distemper was identified as the probable cause of this rapid population decline (Williams et al., 1988). It is now known that black-footed ferrets are susceptible to *Yersinia pestis* (Williams et al., 1994). Consequently it is believed that plague, which was highly prevalent in the prairie dog colonies (Ubico et al., 1988), might also have contributed to the high mortality observed in this population at the time. Following the discovery of an active distemper epidemic in this small isolated population, the remaining black-footed ferrets were progressively captured to protect them from a probable death, and to start a captive propagation breeding program. The last known free-ranging black-footed ferret was captured in March 1987 (Miller et al., 1996).

### 2.1.3 Captive propagation of the black-footed ferret

The first attempt to breed black-footed ferrets in captivity occurred in the early 1970's, when nine animals from the South Dakota population were brought to the U.S. Fish and Wildlife Service Research Center in Patuxent, Maryland (Hillman and Carpenter, 1983). This first tentative breeding effort was not successful, but greatly increased the knowledge on reproductive physiology and captive management of this species.

The capture of the last free-ranging black-footed ferrets in 1987 signalled the beginning of the second attempt at captive breeding. A total of 18 ferrets, seven males and 11 females, caught in Meeteetse, were housed at the Wyoming Game and Fish

research facility in Sybille Canyon for this purpose. However, since some of these 18 animals were known siblings, only 10 ferrets were considered as founders. Only seven of these founders still have descendants in the current captive population (O'Brien et al., 1989). The small size and the prolonged isolation of the Meeteetse population prior its collapse suggest that the founders already had low genetic variability (Russell et al., 1994), which will most likely engender a population with limited genetic diversity. The reproductive failure of some females and the over-representation of one male in the early years of the captive breeding programme also contributed to the decline in heterogeneity of this population (Russell et al., 1994).

Nevertheless, the size of the captive population slowly increased, and at the end of 1997 was composed of more than 380 animals distributed in seven breeding institutions. By the end of 1997, more than 500 black-footed ferrets had been released back to the wild.

## 2.2 Neoplasia in Mustelids

### 2.2.1 Neoplasia in black-footed ferrets

Four of the "South Dakota" ferrets housed at the U.S. Fish and Wildlife Service Research Center died shortly after their capture, due to vaccine-induced distemper (Carpenter et al., 1976). All five remaining ferrets from this group developed adnexal tumours in the caudal part of the body (Table 2.1, page 7).

Three of these five ferrets had two tumours each, and neoplastic disease was believed to be the cause of death in three animals (Carpenter et al., 1981). Attempts at

isolation of viruses from a papillary cystadenocarcinoma of the mammary gland were unsuccessful (Carpenter et al., 1980). It was speculated that this high occurrence of tumours might have been associated with the genetic homozygosity due to inbreeding in this small and isolated population (Carpenter et al., 1981). This hypothesis was supported by the absence of similar conditions in the 200 domestic ferrets (*Mustela putorius furo*) and Siberian polecats maintained as surrogate animals under the same conditions of husbandry (Carpenter et al., 1981). This conclusion, based on five cases, was subsequently frequently cited in the literature as an example of genetic predilection of a population to develop neoplasia consequent to inbreeding.

**Table 2.1** Neoplasms diagnosed in black-footed ferrets from South Dakota. From Carpenter et al. (1981).

| Ferret | Sex | Age | Neoplasms |
|---|---|---|---|
| 1 | Male | 6 | Sweat gland adenocarcinoma of the tail |
| 2 | Female | 6 | Sebaceous gland adenocarcinoma of the tail |
| 3 | Male | 6[a] | Adenocarcinoma of the apocrine gland of the anal sacs |
| | | | Interstitial cell tumour |
| 4 | Female | 12-13[a,b] | Basal cell carcinoma of the tail |
| | | | Papillary cystadenocarcinoma of the mammary gland[c] |
| 5 | Male | 12-13[a,b] | Sebaceous gland adenocarcinoma of the tail |
| | | | Sweat gland adenocarcinoma of the tail |

[a] Estimated age.
[b] Based on today's knowledge, these were probably overestimated.
[c] Also reported in Carpenter et al. (1980).

Reports in the scientific literature of neoplasms in black-footed ferrets from the Meeteetse population are limited to one malignant neoplasm of the nasal epithelium, and one haemangioma, in two animals (Williams, et al. 1988).

### 2.2.2 Neoplasia in Siberian polecats and European polecats

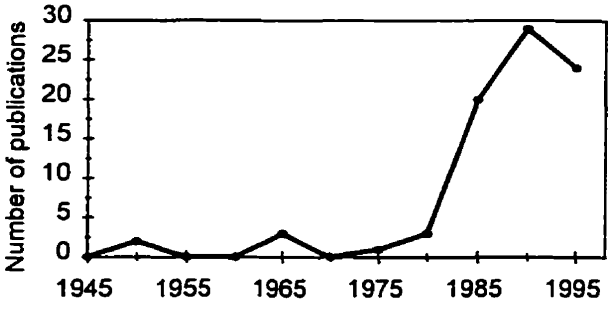
Tumours have been rarely reported in Siberian polecats. Five cases (two hepatic cystadenocarcinomas, two renal tubular neoplasms, and one uterine neoplasm) were described by Williams et al. (1988). Neoplasms reported in European polecats are limited to one unspecified sarcoma (Lombard and Witte, 1959), one sebaceous carcinoma (Rewell, 1949), and three cases of leukosis (Lombard and Witte, 1959; Loupal and Dreier, 1983).

### 2.2.3 Neoplasia in domestic ferrets

Historically, domestic ferrets have been described as a species in which neoplasia rarely occurs (Zwicker and Carlton, 1974; Fox et al., 1986; Hendrick and Goldschmidt, 1987; Meschter, 1989; Rodriguez et al., 1994). Prior to 1980, only 29 cases of neoplasia had been reported in domestic ferrets, in six publications (Symmers and Thomson, 1950; Symmers and Thomson, 1953; Chesterman and Pomerance, 1965; Engelbar and Strasser, 1966; Kenyon and Williams, 1967; Zwicker and Carlton, 1974). Chesterman et al. (1965) suggested that this scarcity of cases implied a genetic resistance of the species to neoplasia, and this was commonly cited subsequently. However, with the increased use of domestic ferrets in research, and with their fast-growing popularity as companion

animals, reports of neoplasms have dramatically increased during the last 15 years, and today neoplasia is a frequent problem in domestic ferrets (Fig. 2.1).

**Figure 2.1** Number of reports published on neoplasia in the domestic ferret.




As of June, 1998, over 80 publications, listing more then 1500 cases of tumours in domestic ferrets, are in the literature (Table 2.2).

**Table 2.2** Neoplasms reported in domestic ferrets, by phenotype.

| Neoplasms | | #[a] | References |
|---|---|---|---|
| Endocrine: | | | |
| Pancreatic islet: | Islet cell tumour | 321 | Lumeij (1987), Kemmerer (1988), Meschter (1989), Beach (1993), Marini (1993), Caplan (1996), Brown (1997), Li (1998). |
| Adrenal gland: | Cortical tumour | 354 | Chesterman (1965), Fox (1987), Paradis (1989), Rosenthal (1993), Lipman (1993), Ackermann (1994), Brown (1997), Weiss (1997), Li (1998) |
| | Pheochromocytoma | 4 | Li (1998) |
| | Myelolipoma | 2 | Li (1998) |
| Thyroid: | Adenocarcinoma | 1 | Li (1998) |
| Other: | Unknown | 1 | Li (1998) |

**Table 2.2** (cont.)

| Neoplasms | | # [a] | References |
|---|---|---|---|
| Haemolymphatic: | | | |
| Lymphoid tissues: | Lymphoma | 292 | Symmers (1953), Rodger (1980), Yanoff (1984), Fox (1986), Dean (1987), Dugan (1989), Flock (1989), Welle (1992), Erdman (1992), Parker (1993), Beach (1993), Li (1995), Erdman (1996), Erdman (1996), Batchelder (1996), Brown (1997), Li (1998) |
| Spleen: | Unknown | 8 | Li (1998) |
| Other: | Myeloproliferative disease | 5 | Chowdhury (1982), Li (1998) |
| | Lymphocytic leukemia | 5 | Kenyon (1967), Altman (1984), Li (1998) |
| | Plasma cell tumour | 2 | Li (1998) |
| | Multiple myeloma | 2 | Li (1998) |
| | Reticuloendotheliosis | 1 | Li (1998) |
| Thymus: | Thymoma | 2 | Taylor (1995) |
| | Erythemic myeliosis | 1 | Li (1998) |
| Integument: | | | |
| Epidermis: | Squamous cell carcinoma | 11 | Symmers (1950), Engelbar (1966), Well (1992), Li (1998), |
| | Basal cell tumour | 42 | Symmers (1953), Well (1992), Parker (1993), Li (1998) |
| | Basosquamous carcinoma | 1 | Li (1998) |
| | Papilloma | 2 | Parker (1993), Li (1998) |
| Sebaceous gland: | Adenoma | 19 | Dillberger (1989), Brown (1997), Li (1998) |
| | Carcinoma | 3 | Li (1998) |
| | Epithelioma | 1 | Li (1998) |
| | Other sebaceous tumour | 2 | Pomerance (1965), Li (1998) |
| Sweat gland: | Adenoma | 3 | Li (1998) |
| | Carcinoma | 5 | Miller (1985), Brown (1997), Li (1998) |
| Adenocarcinoma - apocrine gland - anal sac: | | 1 | Parker (1993) |
| Other: | Mastocytoma | 48 | Poonacha (1984), Dillberger (1989), Stauber (1990), Parker (1993), Brown (1997), Li (1998) |
| | Neuroendocrine tumour | 2 | Li (1998) |
| | Myxoma | 1 | Li (1998) |

**Table 2.2** (cont.)

| Neoplasms | | #[a] | References |
|---|---|---|---|
| | Myxosarcoma | 2 | Chesterman (1965), Li (1998) |
| | Fibrosarcoma | 3 | Parker (1993), Brown (1997), Li (1998) |
| | Maligant histiocytosis | 2 | Dillberger (1989), Li (1998) |
| | Leiomyosarcoma | 1 | Brunnert (1990) |
| | Adenoma unspecified | 5 | Brown (1997), Li (1998) |
| | Fibroma/dermatofibroma | 5 | Brown (1997) |
| | Histiocytoma | 1 | Brown (1997) |
| | Tumour unspecified | 28 | Brown (1997), Li (1998) |
| Digestive: | | | |
| Mouth: | Squamous cell carcinoma | 5 | Brown (1997), Li (1998) |
| | Gingival unspecified | 2 | Li (1998) |
| | Mouth unspecified | 1 | Li (1998) |
| | Salivary gland adenocar. | 1 | Brown (1997) |
| | Fibrosarcoma | 1 | Brown (1997) |
| Stomach: | Adenocarcinoma | 4 | Rice (1992), Fox (1997), Li (1998) |
| | Carcinoma | 1 | Li (1998) |
| | Pylorus, unspecified | 1 | Li (1998) |
| | Tumour unspecified | 2 | Li (1998) |
| Intestine: | Adenocarcinoma | 2 | Li (1998) |
| | Tumour unspecified | 1 | Li (1998) |
| Cecum: | Leiomyoma | 1 | Li (1998) |
| Rectum: | Leiomyosarcoma | 1 | Li (1998) |
| | Tumour unspecified | 3 | Li (1998) |
| Anus: | Squamous cell carcinoma | 2 | Li (1998) |
| | Tumour unspecified | 1 | Li (1998) |
| Liver: | Hepatoma | 1 | Li (1998) |
| | Carcinoma | 1 | Li (1998) |
| | Myelolipoma | 1 | Li (1998) |
| | Tumour unspecified | 9 | Li (1998) |
| | Biliary adenoma | 1 | Li (1998) |
| | Biliary carcinoma | 2 | Symmers (1953), Li (1998) |
| Pancreas: | Adenoma | 6 | Li (1998) |
| | Carcinoma | 9 | Chesterman (1965), Hoefer (1992), Brown (1997), Li (1998) |
| | Tumour unspecified | 8 | Li (1998) |
| Skeletal: | | | |
| Bone: | Osteoma | 4 | Li (1998) |
| | Chondroma | 3 | Li (1998) |

**Table 2.2** (cont.)

| Neoplasms | | #[a] | References |
|---|---|---|---|
| | Fibrosarcoma | 1 | Brown (1997) |
| | Tumour unspecified | 3 | Li (1998) |
| Muscles: | Rhabdomyosarcoma | 1 | Li (1998) |
| Limb or feet: | Tumour unspecified | 6 | Li (1998) |
| | Synovial sarcoma | 1 | Lloyd (1996) |
| Reproductive: | | | |
| Ovary: | Granulosa cell tumour | 2 | Palley (1990), Li (1998) |
| | Luteoma | 1 | Li (1998) |
| | Leiomyoma | 24 | Symmers (1953), Chesterman (1965), Cotchin (1980), Palley (1990), Welle (1992), Beach (1993), Li (1998) |
| | Thecoma | 3 | Chesterman (1965), Palley (1990), Li (1998) |
| | Tumour unspecified | 5 | Brown (1997), Li (1998) |
| Uterus: | Leiomyoma | 1 | Li (1998) |
| Testis: | Interstitial cell tumour | 3 | Dillberger (1989), Li (1998) |
| | Tumour unspecified | 2 | Li (1998) |
| | Sertoli cell tumour | 4 | Palley (1990), Welle (1992), Beach (1993) |
| | Seminoma | 2 | Welle (1992), Brown (1997) |
| Mammary gland: | Cystadenocarcinoma | 2 | Brown (1997), Li (1998) |
| | Complex adenocarcinoma | 2 | Welle (1992) |
| | Tumour unspecified | 1 | Li (1998) |
| Cardiovascular: | | | |
| Spleen: | Haemangiosarcoma | 7 | Parker (1993), Li (1998) |
| Skin: | Haemangioma | 11 | Welle (1992), Parker (1993), Brown (1997), Li (1998) |
| Unknown: | Lymphangioma | 1 | Li (1998) |
| Liver: | Haemangioma/sarcoma | 14 | Cross (1987), Brown (1997) |
| Heart: | Tumour unspecified | 1 | Li (1998) |
| Respiratory: | | | |
| Nose: | Tumour unspecified | 1 | Li (1998) |
| Pharynx: | Tumour unspecified | 1 | Li (1998) |
| Lung: | Adenoma | 1 | Li (1998) |
| Mediastinum: | Tumour unspecified | 4 | Li (1998) |
| Thorax: | Tumour unspecified | 2 | Li (1998) |
| Urinary: | | | |
| Kidney: | Carcinoma | 4 | Symmers (1953), Li (1998) |

**Table 2.2** (cont.)

| Neoplasms | | #[a] | References |
|---|---|---|---|
| | Tumour unspecified | 3 | Li (1998) |
| Bladder: | Transitional cell carcinoma | 2 | Bell (1990), Brown (1997) |
| Prostate: | Carcinoma | 1 | Brown (1997) |
| Nervous: | | | |
| Brain: | Meningioma | 1 | Li (1998) |
| | Astrocytoma | 1 | Li (1998) |
| Adrenal: | Neurofibroma | 1 | Li (1998) |
| Other: | Schwannoma | 3 | Beach (1993), Brown (1997), Li (1998) |
| | Chordoma | 31 | Allison (1988), Dillberger (1989), Herron (1990), Dunn (1991), Roth (1992), Brown (1997) |
| Special sensory: | | | |
| Eye and adnexa: | Eyelid tumour unspecified | 2 | Li (1998) |
| | Eyeball tumour unspecified | 2 | Li (1998) |
| | Orbit tumour unspecified | 2 | Brown (1997), Li (1998) |
| Ear: | Adenoma | 1 | Li (1998) |
| | Papilloma | 1 | Li (1998) |
| | Tumour unspecified | 4 | Li (1998) |
| Head: | Tumour unspecified | 5 | Li (1998) |
| Abdominal cavity: | | | |
| Abdomen: | Leiomyoma | 2 | Dillberger (1989), Li (1998) |
| | Lipoma/sarcoma | 2 | Fuentealba (1995), Li (1998) |
| | Mesothelioma | 4 | Williams (1994), Li (1998) |
| | Mesenteric carcinoma | 1 | Li (1998) |
| | Teratoma | 2 | Beach (1993), Rodriguez (1994) |
| | Carcinomatosis | 11 | Brown (1997), Li (1998) |
| | Tumour unspecified | 40 | Li (1998) |
| Miscellaneous: | | | |
| Unknown location: | Tumour unspecified | 30 | Dillberger (1989), Welle (1992), Brown (1997), Li (1998) |

[a] Total number of cases reported. Due to possible duplicate publication of some case reports in subsequent reviews, this represents a maximum figure.

Only one of these publications contains enough information on the population at

risk to allow estimation of the incidence. Thirteen cases of tumours are described in two breeding colonies of domestic ferrets. Seven cases were diagnosed during an 8 year period from a population composed on average of 46 ferrets. The other six cases were seen over a 9 year period in a group of approximately 200 ferrets (Beach and Greenwood, 1993). Based on these numbers the annual incidence of neoplasia in these two populations can be estimated at approximately 2% and 0.3% cases per year respectively.

A survey published by Li et al. (1998) gathered from the Veterinary Medical Data Base at Purdue University gave the rate of neoplasia in domestic ferrets presented at several North American veterinary teaching hospitals. A total of 639 tumours were diagnosed in 574 (12% ) of the 4774 domestic ferrets included in this study (Li et al., 1998).

Surveys of neoplasms encountered in exotic animal hospitals (Welle and Gobel, 1992; Brown, 1997), submitted to a diagnostic laboratory service (Dillberger and Altman, 1989), diagnosed in two colonies of ferrets used for research (Beach and Greenwood, 1993), or gathered from several universities (Li et al., 1998), provide the relative frequencies of different neoplasms affecting the domestic ferrets. Table 2.3 (page 15) lists the principal tumours encountered in these studies.

The disparities observed among these four studies might be explained, at least in part, by the differences among the populations. Cases of insulinomas and adrenocortical tumours, both associated with well-recognised clinical syndromes in domestic ferrets, are less likely to be submitted for post mortem diagnosis, and will therefore be under-represented in surveys from diagnostic laboratories, such as Dillberger et al. (1989).

Nevertheless, these discrepancies between studies also suggest a possible geographical clustering of cases, which could be associated either with lineage, or with exposure to unknown carcinogenic factors.

**Table 2.3** Comparison of four different surveys for neoplasms in domestic ferrets. Only the five more common type of tumours are presented.

| **Authors** | **Dillberger (1989)** | **Li (1998)** | **Brown (1997)** | **Beach (1993)** | **Welle (1992)** |
|---|---|---|---|---|---|
| Geographic location | USA | USA | USA | UK | GER |
| Total number of cases | 12 | 639 | 380 | 13 | 25 |
| | Number of cases (relative frequency %) | | | | |
| Insulinoma | 0 (0) | 139 (21.7) | 114 (30.0) | 1 (7.7) | 0 (0) |
| Adrenocortical tumour | 0 (0) | 107 (16.7) | 97 (25.5) | 0 (0) | 0 (0) |
| Lymphoma | 1 (8.3) | 76 (11.9) | 89 (23.4) | 6 (46.2) | 5 (20.0) |
| Mast cell tumour | 1 (8.3) | 17 (2.7) | 17 (4.5) | 0 (0) | 0 (0) |
| Sebaceous tumour | 3 (25.0) | 6 (0.9) | 10 (2.6) | 0 (0) | 0 (0) |

Interestingly the vast majority of cases of neoplasms in domestic ferrets have been reported in North America. On the 82 reports retrieved, 64 were from North America, and only 10 from Europe (the geographical origin for the eight other reports was unknown). Due to possible duplicate publication of some case reports in subsequent reviews, the actual number of cases is difficult to evaluate. However, a maximum of only 66 cases has been described in Europe compared to more than 1500 in North America. Even if a selection bias toward American publications exists, the larger population at risk in North America, and possible bias associated with differences between American and European populations might partially account for this geographic difference, the under-

representation of European and Australian cases suggests that the North American ferrets either have a higher susceptibility to neoplasia, or are more exposed to carcinogenic factors.

North American ferrets may be genetically predisposed to certain types of tumour (Brown, 1997). Because of the high demand for ferrets in the United States, breeding facilities are more likely to tolerate inbreeding. Risk of developing familial diseases will therefore be higher, compared to Europe or Australia, where the demand for ferrets is lower (Brown, 1997). The virtual absence in Europe of some of the most common neoplasms in North American ferrets, such as the adrenocortical tumours, basal cell tumours, chordoma, and mast cells tumours, suggests the existence of linages predisposed to specific phenotypes of neoplasia. Early neutering, routinely performed in North America, has also been suggested as a potential explanation for the relatively high number of adrenocortical tumours encountered in North American ferrets (Rosenthal et al., 1993). Other predisposing factors proposed include lack of natural photoperiod, differences in diet, and possibly infectious agents (Brown, 1997).

Numerous cases of hyperadrenocorticism associated with adrenocortical tumours have been published (Table 2.2, page 9). A comprehensive review of this syndrome is given by Rosenthal et al. (1993). The relatively recent appearance of this syndrome (only one case was published before 1987), suggests a potential association with new management techniques. Rosenthal et al. (1993) speculated that premature gonadectomy in pet ferrets might play a role in the development of these neoplasms. The emergence of a lineage with an increased susceptibility to adrenocortical tumours would also explain

this uneven distribution in time. However, this hypothesis is not supported by any reports of familial clustering.

Insulinomas, only reported since 1984 (Kaufman et al., 1984), are today probably the most common neoplasms encountered in domestic ferrets in North America (Brown, 1997). Two case series examined the clinical course, the pathological findings, the therapeutic regimens, and the survival rate associated with this neoplastic syndrome (Ehrhart et al., 1996; Caplan et al., 1996). As for adrenocortical tumours, insulinomas have only been common since domestic ferrets became abundantly available as companion animals.

Lymphomas also have been commonly described in domestic ferrets [for a review of clinical and pathological findings see Erdman et al. (1996)]. An interesting feature of these neoplasms in ferrets is their relative tendency to be gathered in clusters (Rodger, 1980; Erdman et al., 1996; Batchelder et al., 1996), and an infectious etiology has been suggested by several authors to explain this. Feline leukaemia virus (Dean, 1987; Rodger, 1980), and Aleutian mink disease virus (Kenyon and Williams, 1967), were first proposed as potential etiologic agents, but subsequent studies did not support the implication of these viruses (Erdman et al., 1996; Brown, 1997). The successful transmission of lymphomas with cell-free inoculates from a clinical case, and the detection of retroviral particles in spleen cells cultured from an affected ferret, implicate a retrovirus in the etiology of this tumour (Brown, 1997). One report of clustered cases in consanguineous ferrets also suggests a possible role of an inherited genetic trait in the development of these neoplasms (Erdman et al., 1996), although contagion or vertical

transmission of a virus within a family might also explain such a pattern.

The potential link between gastric adenocarcinoma and infection by *Helicobacter mustelae* represents another example of possibly infectious etiology of neoplasms in the domestic ferret (Rice et al., 1992; Fox et al., 1997), although such tumours are relatively rare (Table 2.2, page 9).

Genetic predisposition has been suggested as a potential explanation for the unusually high incidence of hepatic vascular neoplasms in a colony of ferrets used for research in reproduction (Cross, 1987); for the relatively high number of reports of chordomas, an uncommon neoplasm in domestic animals [for a comprehensive review see Dunn et al. (1991)]; and for a high occurrence of ovarian leiomyoma in a closed colony of albino ferrets issued from eight founders (Cotchin, 1980).

### 2.2.4 Neoplasia in other members of the Mustelidae

The vast majority of neoplasms reported in the literature on mink (*Mustela vison*), were derived from a group of animals used in studies on slow viral diseases. In one report, 235 nonhematopoietic/nonlymphoreticular neoplasms were detected in nearly 5000 mink examined at necropsy over a 20-year period (Hadlow, 1984). A complete description has been published for three types of these neoplasms. Vertebral chordomas were reported in two mink (Hadlow, 1984), tumours of the carotid body were described in 15 mink aged between 6 and 11-yr-old (Hadlow, 1986), and a series of 60 cases of carcinomas originating from the apocrine gland of the anal sacs was also reported (Hadlow, 1985). The latter tumours, based on postmortem examination of 870 mink,

occurred in animals between 5 and 11-yr-old, and all but one affected females (Hadlow, 1985). This unusual number of cases was believed to be due to the advanced age of the mink in this population. Other tumours in mink already affected by a carcinoma of the apocrine gland of the anal sacs included 19 cases of lymphoma, one adrenocortical adenoma, one hepatocellular carcinoma, one leiomyoma of the jejunum, one fibrosarcoma of the face, one carcinoma of the exocrine pancreas, and one insulinoma (Hadlow, 1985).

Only two other cases of neoplasia have been described in mink: a squamous cell carcinoma on the tail of a male (Gorham and Quortrup, 1948), and an adenocarcinoma, probably uterine in origin (Graves, 1937).

A series of neoplasms, including four cases of Hodgkin's disease, were diagnosed in 11 of 66 captive skunks (*Mephitis mephitis*), and in one of 86 wild skunks necropsied (Smith and Barker, 1983). Tumours reported included pulmonary adenocarcinoma, pulmonary adenomatosis, undifferentiated adenocarcinoma, renal adenocarcinoma, thyroid adenoma, interstitial cell tumour, pheochromocytoma, pinealoma, squamous cell carcinoma, and spindle cell sarcoma (Smith and Barker, 1983).

Other cases of neoplasia in mustelids included: a biliary adenocarcinoma in a wild British otter (*Lutra lutra*) (Wells et al., 1989); a thyroid adenoma in a captive fisher (*Martes pennanti*) (Carlson and Nielsen, 1985); a carcinoma of type II pneumocytes in a captive striped skunk (Miller et al., 1985); a "hepatic vascular neoplasm" in a wild badger (*Taxidea taxus*) (Paget, 1978); an ovarian teratoma in a captive spot-neck otter (*Hydrictis maculicollis*) (Zachary and McDonald, 1981); and a biliary adenocarcinoma, an uterine

leiomyoma, and a pheochromocytoma in a captive sea otter (*Enhydra lutris*) (Stetzer et al., 1981).

## 2.3 Etiology and epidemiology of neoplasia in populations

### 2.3.1 Generalities - carcinogenesis

The British oncologist Sir Rupert Willis (1973) defined a neoplasm as "an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of the normal tissues and persists in the same excessive manner after cessation of the stimuli which evoked the change". The uncoordinated cellular proliferation involved in neoplasia is based on alterations in the control mechanisms regulating cell division and apoptosis. The dysfunction of these cellular and extracellular control mechanisms is usually due to nonlethal genetic damage to growth-promoting proto-oncogenes or growth-inhibiting cancer suppressor genes. These mutations result either in the activation of proto-oncogenes in oncogenes or in the inactivation of cancer suppressor genes (Cotran et al., 1994).

In contrast to proto-oncogenes, for which the mutation of one of the two alleles is sufficient for their transformation, inactivation of both normal alleles is required in cancer suppressor genes for the development of a neoplasm. Cancer suppressor genes are therefore frequently referred to as recessive oncogenes. These mutations can either be acquired in somatic cells or inherited in the germ line (Cotran et al., 1994).

Any regenerative, hyperplastic, and dysplastic proliferation would increase the potential clonal expansion of mutated cells. Frequent mitoses could "fix" the mutation by

interfering with the cellular mechanisms of DNA repair. Processes associated with increases in cellular turnover are hence considered as tumour promoters. Endometrial hyperplasia induced by exogenous oestrogens, and cellular regeneration observed in cirrhotic liver, are examples of such processes (Cotran et al., 1994).

### 2.3.2 Acquired mutations following exposure to carcinogens

Acquired mutations in somatic cells may be caused by a variety of carcinogens, including ionizing radiation, such as ultraviolet light (UV), x-rays, etc.; carcinogenic xenobiotic chemicals, including dietary carcinogens; and infectious agents, mainly viruses. The degree of exposure of a population to these mutagenic agents will therefore influence the incidence of specific neoplasms encountered in this population. The list of chemical and physical carcinogenic agents associated with increased incidence of cancers, either in natural or experimental conditions, is extensive [for reviews see Harris (1991) and Pitot (1985)]. Examples of these carcinogens are listed in Table 2.4.

**Table 2.4** Examples of neoplastic diseases associated with environmental carcinogens.

| Species | Carcinogen | Tumours induced | References |
|---|---|---|---|
| Wild fish | PAH[a] | Hepatic tumours | Harshbarger et al. (1990) |
| Human | Asbestos | Mesothelioma | Wagner (1984) |
| Various | Aflatoxins | Hepatic tumours | Busby et al. (1984) |
| Various | Sun exposure (UV) | Skin tumours | Cleaver (1993) |
| Human | Aromatic amines | Bladder cancer | Kleinfeld et al. (1966) |

[a] Polycyclic aromatic hydrocarbons

Several DNA (herpesvirus, papillomavirus) and RNA (retrovirus) viruses can induce alterations in host DNA, while others, such as hepadnaviruses, may act as promoters in the development of cancer. It is believed that 15% to 25% of all cancers in people are linked to viral infections (zur Hausen, 1996). Burkitt's lymphomas caused by Epstein-Barr virus, liver cancer associated with hepatitis B virus, and cervical cancer associated with papillomaviruses, are well-documented examples of etiologic associations between cancers and viruses in people (zur Hausen, 1996). Numerous viral-induced neoplastic diseases also have been described in a variety of animal species (Klein, 1985). A small sample of examples is given in Table 2.5.

**Table 2.5** Examples of viral-induced neoplastic diseases in animals.

| Species | Virus | Neoplasms induced | References |
|---|---|---|---|
| Cat | Retrovirus (FeLV) | Lymphoma | Jarret et al. (1969) |
| Walleye | Retrovirus | Cutaneous sarcoma | Martineau et al. (1990) |
| Cattle | Papillomavirus | Gastric neoplasms | Campo (1987) |
| Mouse | Mammary tumour virus | Mammary neoplasms | Gross (1984) |
| Poultry | Herpesvirus (Marek's) | Lymphoma | Biggs (1967) |
| Leopard frog | Herpesvirus (Lucké's) | Renal carcinoma | Granoff (1973) |
| Groundhog | Hepadnaviruses | Hepatoma | Tyler et al. (1981) |

### 2.3.3 Heritability of neoplasia

Neoplasia is a multifactorial disorder that may be influenced by genetic factors (Cooper, 1993). Rare forms of cancers in people have been associated with single-gene

disorders (Philippe, 1989). These familial cancers are associated with inherited mutations in the germ line that are transmitted from parent to child. Individuals affected by these genetic syndromes will frequently develop multiple independent tumours at an early stage of life [for a review see Philippe (1989)]. Li-Fraumeni familial cancer syndrome, and familial retinoblastoma are well-known examples of familial cancers. The familial transfer of these single-gene disorders should be in accordance with Mendelian inheritance (Nicholas, 1996). Most of these syndromes seem to be transmitted in a genetically dominant fashion, but autosomal recessive inheritance has been speculated for clusters of neoplasia in families with consanguineous unions (Lebel and Gallagher, 1989). Cancers with recessive patterns of inheritance have also been described in certain inbred strains of mice (Slye, 1922).

Genetic predispositions to cancer not due to a single gene are more common. Familial predisposition to common sporadic neoplastic diseases, such as lung, breast and colon cancers, has been described frequently in people (Philippe, 1989). Similar predispositions seem to occur in animals, where the occurrence of neoplasia varies from species to species. Variation in tumour occurrence is also observed among breeds of dogs (Hayes, 1981), and loss of genetic heterogenicity in these breeds may be a factor predisposing to neoplasia (Ubbink et al., 1992). Examples of possible familial or genetic predispositions in zoo animals include cholangiocellular carcinomas in sloth bears (Montali, 1980), and ovarian tumours in maned wolves (Munson and Montali, 1991).

### 2.3.4 Neoplasia and other host factors

In females, reproductive history seems to affect the occurrence of "endocrine-driven" tumours. In women, for example, late age at first pregnancy is associated with breast cancers, and low parity with ovarian cancers (Doll and Peto, 1981).

In women, obesity is also a significant predisposing factor for endometrial cancers and for breast cancers (Cooper, 1993), and it is now well known that feeding regimens (*ad libitum* vs rationing) greatly affect the occurrence of cancer in laboratory animals (Munro et al., 1995).

Since the effects of mutagenic insults are cumulative, the risk of developing cancer is expected to increase with age. Consequently, age is often the most significant determinant for neoplasia, and the life span of a population will affect the incidence of cancer. The rarity of neoplasia in free-ranging wild animals is probably a consequence of their relatively limited life span, compared to animals kept in captivity (Fowler, 1987).

# 3 MATERIALS AND METHODS

## 3.1 Objectives

The general objective of this project was to better characterize the neoplastic conditions encountered in the captive population of black-footed ferrets. We used retrospective data to describe the occurrence of the different types of neoplasms in this population in relation to their occurrence in other populations of animals, and the histomorphology and biological behaviour of these neoplasms is described. We also had as an objective the evaluation of possible associations between neoplastic diseases and various host and environmental factors, and to explore familial relationships among black-footed ferrets affected by neoplasia as a whole, and by specific neoplastic conditions. The outcomes of this study could then be applied to evaluation of the implications for the captive breeding program of neoplasia in black-footed ferrets, and to the development of strategies to mitigate the impact of neoplasia, if appropriate and feasible.

## 3.2 Study population

The study population was composed of all the black-footed ferrets listed in the studbook of the Species Survival Plan at the end of the year 1996. This studbook was managed by Mr. Paul Marinari at the USFWS National Black-Footed Ferret Conservation Center, and catalogues all the black-footed ferrets that have been in captivity since the establishment of the "Meeteetse" captive breeding program. A total of 1872 animals,

maintained in seven different breeding centres (Appendix I), were listed in this studbook at the end of 1996. Table 3.1 summarizes the status of these animals as of May 15, 1998 for animals housed at the Toronto Zoo, and November 1, 1997 for animals at the other institutions. This includes only animals born up to and including 1996 following their fate into the subsequent years.

**Table 3.1** Status of the black-footed ferrets listed in the 1996 studbook (study population).

| Status | Number of ferrets | (male.female.unknown) |
|---|---|---|
| Died before 1 year of age | 710 | (148.122.440) |
| Died after 1 year of age | 227 | (125.102.0) |
| Released back to the wild | 548 | (305.235.9) |
| Still alive | 386 | (155.231.0) |
| **Total** | **1872** | **(733.690.449)** |

### 3.3 Selection of study animals (study group)

For this study, the population at risk was determined to be all the ferrets born up to and including the year 1996, that survived for at least one year. This decision was motivated by the fact that upon preliminary evaluation of the data, neither neoplasms nor hepatic cysts were detected in immature animals. Because neoplasms could remain undetected in a live animal for a period of time, only ferrets that were necropsied were included in this study. Of the 227 ferrets meeting these two criteria, 43 animals (19%)

were eliminated, either due to insufficient information, or due to inability to retrieve the full necropsy report. A total of 184 ferrets was therefore included in this study, and these will hence forth be referred to the "study group". We are confident that this subset is representative of all the adult ferrets which have died over one year of age since the distribution of age, inbreeding coefficient, gender, and time spent at each institution does not differ from the group as a whole. Clinical files and postmortem reports were retrieved for each animal of the study group from institutions participating in the breeding program, and from seven different diagnostic laboratories, respectively (Appendices I and II).

### 3.4 Collection of data and retrieval of archived materials

The most consistent and reliable source of demographic information on the captive population of black-footed ferrets was the Species Survival Plan Studbook. Parameters such as identification, sex, date of birth, date of death, inbreeding coefficient, sire, and dam, were directly collected from this studbook. These were used to calculate additional variables such as age at death, time spent at each institution, familial relationship (sibling, offspring), and reproductive data (number of litters, lactation, age at first parturition, etc.). Postmortem reports were reviewed for macroscopic and/or microscopic description of neoplasia (including metastases) and hepatic cysts. Information on the clinical history was also commonly available on these postmortem reports. Clinical files, retrieved from the institutions participating to the breeding program, were the third source of information. Due to the lack of systematic compilation

of *antemortem* parameters, and due to the marked variation as far as quantity and quality of the information recorded, retrieval of data from these files was arduous and incomplete. Consequently, information extracted from the clinical files could not be included in the epidemiologic analysis, but was used in the description of the clinical manifestations of each neoplastic syndrome.

All retrievable paraffin-embedded blocks of neoplastic tissues (88), kidney (114), and liver (115) were recovered and examined. Reports and tissues from surgical biopsies from animals included in the study group were also examined. Tissues were exported from the United States of America into Canada under the International Trade in Endangered Species (CITES) regulations (permit number: CA-CW-IM-0016-98). Sections 6 μm thick were cut from each block and stained with haematoxylin and eosin (Luna, 1968). Each section was then examined by the same pathologist (S.L.) by light microscopy. Brown and Brenn, Gram, and Perl's iron stains were used on selected sections of liver, and Periodic Acid Schiff staining was used in selected peri-anal tumours to assess the presence of mucus (Luna, 1968).

A total of 62 variables was logged in a spreadsheet (Qattro Pro®), using one line per animal, and one column per variable (Appendixes VI and VII). A description of these variables is given in Appendix III; they involved seven biologic parameters, nine familial parameters, five female reproductive parameters, 39 pathology parameters, and nine management parameters.

## 3.5 Classification of neoplastic and dysplastic lesions

Slides from archived tissues were all examined once in order to evaluate the range of lesions present. Neoplasms were classified during a second histologic evaluation following consultation with selected references (Hampe and Misdorp, 1974; Bennington and Beckwith, 1975; Sass, 1986; Craig et al., 1988; Moulton, 1990a).

### 3.5.1 Lesions of the biliary tree

Cholangiohamartoma-like lesions (Redston and Wanless, 1996) were circumscribed clusters of aberrant bile ducts embedded in a fibrous stroma with limited inflammation. Biliary cysts were segmental cystic dilations of the biliary tree, with or without non-branching intraluminal fibrous septa. The term biliary cystadenoma was used for polycystic tumours showing obvious proliferation of intraluminal branched septa or papillae, lined by one or two rows of epithelial cells, forming a "spider web" pattern. A diagnosis of biliary cystadenocarcinoma was made for tumours displaying intracystic papilliform proliferation of the lining epithelium, forming intraluminal neoplastic growths, and with cytologic characteristics or behaviour indicative of malignancy (Cotran et al., 1994).

### 3.5.2 Renal tubular neoplasms

The discrimination between renal adenomas and renal adenocarcinomas is arbitrary (Bennington and Beckwith, 1975; Moulton, 1990a). The renal tubular neoplasms observed in the black-footed ferrets were representative of a continuum of

lesions, and since objective criteria to predict malignancy could not be determined, no attempt was made to classify them as benign or malignant. They all were included in the general category of "renal tubular neoplasm".

### 3.5.3 Sweat gland and preputial apocrine gland neoplasms

Discrimination between adenomas and adenocarcinomas of the sweat gland, and of the preputial apocrine gland, was not always clear-cut. The presence or absence of local invasion, and the degree of cellular atypia were the two main criteria used. Adenomas were small well-circumscribed masses composed of well-differentiated hyperplastic apocrine glands. Adenocarcinomas were also usually well-differentiated, but showed some degree of invasion, and a higher level of anaplasia.

### 3.5.4 Neoplasms of the mammary gland

Neoplasms of the mammary gland were classified according to the World Health Organization (Hampe and Misdorp, 1974).

### 3.5.5 Neoplasms of the apocrine gland of the anal sacs

All neoplasms of the apocrine gland of the anal sacs were classified as adenocarcinomas, based on their cytologic characteristics and their invasive behaviour.

### 3.5.6 Other neoplasms

Other neoplasms were classified according to their histologic morphology as

described by Moulton (Moulton, 1990a).

## 3.6 Histological assessment of neoplastic lesions

The following histologic features were evaluated for each tumour.

### 3.6.1 Degree of invasion

The degree of invasion of the adjacent tissue by the neoplastic cells was classified as absent (0), mild (1), moderate (2), or severe (3).

### 3.6.2 Necrosis, fibrosis and osseous metaplasia

Necrosis in the neoplastic tissue, the induction of fibrosis, and the presence of osseous metaplasia associated with the neoplasms were graded as absent (0), mild (1), moderate (2), or severe (3).

### 3.6.3 Cellular organisation

The cellular organisation of the neoplastic cells was defined using descriptive terms, like "tubular", "papillary", "acinar" and "solid", as described elsewhere (Moulton, 1990a).

### 3.6.4 Degree of cellular anaplasia

The degree of cellular anaplasia was subjectively assessed using four degrees of severity, 0 being the lowest degree of cellular anaplasia and 3 the highest. Criteria such

as shape and size of the cells, nuclei, and nucleoli, cytoplasmic appearance, and presence of cellular abnormalities were used for this assessment (Moulton, 1990a).

### 3.6.5 Degree of anisokaryosis

The ratio between the diameter of the largest and the smallest nuclei of neoplastic cells in a section was used to describe the degree of anisokaryosis (eg. 2-fold; 4-fold).

### 3.6.6 Mitotic index

The mitotic index was defined as the highest number of mitoses observed in one microscope field at a magnification of 400 X (Objective Olympus DPlan 40, with an Olympus BH-2 microscope). Between 10 and 15 fields were screened to establish this estimate.

## 3.7 Lead in kidneys

Since renal carcinomas have been described in free-ranging rats exposed to lead (Kilham et al., 1962), the level of this potential environmental or food contaminant was assessed in the kidney of four black-footed ferrets (SB#: 86, 101, 173, and 393). Three of these animals had renal tubular neoplasms, and one was exempt. This evaluation was performed (Animal Health Laboratory, Guelph, Ontario, N1H 6R8) on frozen kidney using microwave assisted nitric acid digestion with flame atomic absorption spectroscopy analysis. The detection limit of this method is 5 ppm.

## 3.8 Epidemiology

### 3.8.1 Definition of the events of interest

The following events of interest were used in the epidemiological analysis (for definition see pathology parameters in Appendix III): "cysts", "bile_tumour", "renal", "sweat", "apo_anal", "mamm", "apocrin", "epithel", and "tumour".

### 3.8.2 Determination of prevalence at death and incidence of neoplasia in the study population

Prevalences at death of each event of interest in the captive population of black-footed ferrets were evaluated as follows:

$$Prevalence = \frac{Animals\ with\ event\ of\ interest}{Study\ group}$$

where the study group was composed, as previously defined, of all dead ferrets older than a year of age for which sufficient postmortem data were available (Martin et al., 1987).

Crude annual rates for each event of interest per 100,000 animals were calculated as follows:

$$CAR = \frac{No.\ new\ cases\ with\ events\ diagnosed\ during\ the\ IT}{No.\ of\ ferret\text{-}years\ of\ observation} \times 100,000$$

The *CAR* is the crude annual rate of neoplasia per 100,000 animals. The *No. new cases with events diagnosed during the IT* is the number of ferrets with the event of interest that died during the interval of time (IT); in this case 1 year. The *No. of ferret-years of observation* is the sum of the lengths of time during the specified interval that each

member of the study population was alive and under observation (ie. fractions of years per ferret, up to 1 year) (Martin et al., 1987). Each interval of time was set to one year starting on November 1 of the previous year and ending on October 31 of the current year. It should be emphasised that the entire study population (Studbook, 1996), and not only the study group, was used to determine crude annual rates and other measures of incidence. For the calculation of crude annual rates for 1997, ferrets born during the summer 1997 were added to the study population.

One of the objectives of this project was to determine if the occurrence of neoplasia in the captive population of black-footed ferrets is unusually high. To answer this question, the situation observed in our population has to be compared with the occurrence of neoplastic diseases in other populations. Obviously, the best population to use for that would be another group of black-footed ferrets. Unfortunately, due to the very small size of the only other population of black-footed ferrets studied (South Dakota), populations of other species had to be used for comparison.

Human populations probably have the best potential for comparison. In contrast to most other species of animals, information on the incidence of neoplasia in people is readily available. In addition, human populations in developed countries, and the captive population of black-footed ferrets resemble each other somewhat. Mortalities in these populations are largely due to natural causes (no culling), and the disease surveillance effort is similar, although autopsies are not performed on every human death. Data for human populations were obtained from the National Cancer Institute of Canada (Anonymous, 1997b), and from the SEER Cancer Statistics Review (Anonymous,

1997a). Comparisons will also be made with all data found for zoo animals (Effron et al., 1977; Lombard and Witte, 1959; Appleby, 1969; Montali, 1980), and mustelids (Beach and Greenwood, 1993; Priester and McKay, 1980; Schneider and Hunter, 1993; Beek et al., 1990; Hadlow, 1984), and with selected data for domestic animals (Priester and McKay, 1980), mice (Sass, 1986), and rats (Chandra et al., 1992) reflecting large databases. Comparisons will also be performed for renal tubular neoplasms, and for mammary gland neoplasms.

To compare CAR in black-footed ferrets with CAR in other species, all CARs were adjusted to the human life span. The life span adjusted annual rates (LSAAR) in defined as follows:

$$LSAAR = CAR\ species\ X\ \frac{species\ life\ span}{human\ life\ span}$$

The following life spans were used: human - 85 years; dog, cat and ox - 15 years; horse - 25 years; domestic ferret and black-footed ferret - 8.5 years. The choice of these life spans was based on the age distributions of the study group and of each comparative species (Priester and McKay, 1980; Anonymous, 1997b).

### 3.8.3 Determination of "symptomatic ratios" and "fatality ratios"

"Symptomatic ratios" and "fatality ratios" were calculated for the most common conditions. The "symptomatic ratio" represents the percentage of cases associated with clinical signs, and the "fatality ratio" represents the percentage of animals affected by the

condition that died or were euthanatized due to this condition.

### 3.8.4 Statistical analysis

For each events of interest, the study group was divided into two groups, those with the event at post mortem and those without. Two-sample t-tests (Moore and McCabe, 1993), were used to assess significant differences between these groups in age, date of birth, date of death, inbreeding coefficient, percentage of life spent at each institution, status of relatives as far as events of interest, and reproductive performance for each ferret of the study group. The chi-square test was used to determine if there was a significant difference in the two groups with respect to gender.

Multivariate logistic regression, using host and environmental variables listed in Appendix V, was used to investigate the relationship of these variables as factors in the probability of having the event of interest. A model was built for each event of interest listed in section 3.8.1. Independent variables were initially screened for statistical association with each event of interest. Variables with p-values $\leq 0.25$ were then included step-wise in each model until no further significant improvement of the model was seen. In order to assess the strength of this association, odds ratios (OR) were computed for significant variables. The predetermined intervals for the continuous variables are identified in the footnotes of Table 4.10 (page 85).

The relationship between the disease status of the parents and of their offspring was evaluated to explore possible patterns of Mendelian inheritance for each syndrome. Due to the absence of neoplasms in animals younger than 3 years of age, only ferrets that

were at least 3-yr-old at death and with both parents in the study group (a total of 116 animals) were included in this analysis. For each event of interest, animals were divided into three groups: no parents affected, one parent affected, and both parents affected by the event of interest. The percentages of offspring affected by the event of interest in each of these three groups were then compared, using pair-wise chi-square or Fisher's exact tests to determine if significant differences were present between these groups with respect to the ratio of affected offspring. A one-way analysis of variance (ANOVA) was performed for each event of interest to evaluate the differences in mean age at onset between animals having one, two, or three parents affected.

Finally, the strength of association between age and total number of neoplasms per animal was measured by Spearman's correlation coefficient. A significance level of $p \leq 0.05$ was used in all statistical tests. Statistical analyses were performed using SAS software (SAS Institute Inc., Cary, NC, USA).

# 4 RESULTS

## 4.1 Clinical and postmortem findings of biliary cysts and neoplastic conditions

### 4.1.1 Biliary cysts

*Clinical and macroscopic findings*

Biliary cysts were detected in 121 of the 184 ferrets in the study group. These cysts were seldom associated with clinical signs, and in some animals, regularly monitored, their size remained unchanged for several months. Anorexia and abdominal distension were the most common clinical signs reported, and were seen mainly in ferrets with secondary bacterial infection of the cysts.

These cysts ranged in size from microscopic to large coalescing structures almost completely replacing affected hepatic lobes. They generally were multiple, single cysts being observed in only 13 animals. Cysts were usually visible from the external surface of the liver, were delimitated by a thin translucent wall, and compressed or replaced the adjacent parenchyma (Fig. 4.1A). Large cysts were usually formed by one chamber, and smaller cysts were often multiloculate. They were usually filled with an abundant, slightly viscous clear to light yellow fluid. Communication between these cysts and the biliary tree was not detected. Rupture of biliary cysts into the abdominal cavity occurred occasionally. Cysts filled with a thick grey/brown pasty to frankly purulent exudate were described in 12 ferrets, and foul-smelling thick exudate was observed in the abdomen in six of these cases.

*Histological features*

A total of 81 biliary cysts was available for histological evaluation. This lesion

was microscopically characterized by variously-sized intrahepatic uniloculate (in 19 cases) or multiloculate (in 62 cases) cystic structures. Most cysts were subcapsular, and they often protruded from the hepatic surface. They mildly compressed the adjacent hepatic parenchyma, and were delimitated by a poorly cellular layer of fibrous tissue of variable thickness (Fig. 4.1B). This fibrous capsule occasionally contained capillaries, and was lined, on its internal aspect, by usually one, but occasionally two rows of epithelial cells. These cells were usually flattened, but cuboidal epithelial cells were also frequently observed, especially in the smaller cysts. The lining cells were homogeneous in size and shape, possessed densely acidophilic cytoplasm, an ovoid variously sized often vesicular nucleus, and resembled epithelial cells of the bile ducts. Mitotic figures were not detected. The lumen of these cysts were usually empty, but occasionally contained a pale acidophilic amorphous material, and was filled with numerous degenerate neutrophils in six cases. Occasional aggregates of Gram negative bacterial colonies were detected in the purulent exudate in three of these cases.

Various degrees of cholangiocellular hyperplasia were observed in 73 of the 81 livers with biliary cysts examined. These proliferative lesions were characterized by aggregates of immature cholangiolar cells occasionally forming rudimentary ductules (Fig. 4.2A). Clusters of mildly dilated disorganised biliary ducts embedded in a fibrous stroma (Fig. 4.2B) were seen in 17 of the 115 livers examined (14.8%). These clusters of hyperplastic biliary elements were morphologically similar to cholangiohamartomas described in people, also known as von Meyenburg complexes (Klatskin and Conn, 1993). These cholangiohamartoma-like lesions were only detected in ferrets older than 5

years of age, and only in animals with biliary cysts. The hepatic parenchyma frequently contains clusters of poorly differentiated epithelial cells, believed to be hepatocellular precursors (Fig. 4.2C). Aggregates of haematopoietic precursors, and of lymphocytes and neutrophils, were also commonly infiltrating the hepatic parenchyma surrounding these biliary cysts.

### 4.1.2 Biliary cystadenoma and biliary cystadenocarcinoma

*Clinical and macroscopic findings*

Lethargy, abdominal distension, ascites, anorexia, palpable abdominal masses, and weight loss were the most common clinical signs seen in ferrets with tumours of the biliary tree. Biliary cystadenocarcinomas were characterized by sudden evolution of the clinical signs, and fast growth. As an example, no abnormalities were reported during the physical examination of one of the affected ferrets four months prior to its death from a large biliary cystadenocarcinoma with diffuse abdominal carcinomatosis (SB# 448).

Biliary cystadenomas were grossly similar to the multilocular biliary cysts. Numerous cysts were described in all 13 cases for which postmortem reports were available, but grossly detectable hepatic masses were reported in only two cases. These irregular, poorly defined masses were composed of a moth-eaten spongy grey tissue. Abdominal distention and ascites were reported in two and three cases respectively.

Abdominal distention and moderate to marked ascites were observed respectively in five and six ferrets affected by biliary cystadenocarcinomas (of a total of 23 postmortem reports reviewed). In all cases, numerous cystic structures were in the liver.

Grossly visible solid masses were described in the liver of 15 of the 23 cases reviewed. These irregular and multilobulated masses were infiltrating the hepatic tissue, and were formed by numerous variously coloured (from white to dark red) friable nodules and cystic structures (Fig. 4.4A). Adhesions were occasionally observed between these masses, the abdominal viscera, and the omentum. Location of the primary tumour was available in eight cases: the left lateral and right lateral hepatic lobes were affected in two cases each, both right lateral and right medial lobes were involved in one animal, and numerous masses affecting all lobes were described in three cases. Abdominal carcinomatosis was reported in 12 of the 23 cases. This was characterized by numerous randomly dispersed variously-sized firm white to red/brown masses attached to the peritoneum, mesentery and serosa of various viscera such as intestine, liver, spleen, and gallbladder. On cut surfaces these often pedunculate nodules frequently appeared bony.

*Histological features*

Biliary cystadenomas were always associated with biliary cysts, and were mainly characterized by poorly defined intracystic or pericystic complex networks of variously sized and shaped ductular microcavities separated by a thin connective stroma (Fig. 4.3A). These connective tissue septa frequently formed intraluminal projections, and were lined on both sides by one row of flattened to cuboidal epithelial cells (Fig. 4.3B). These epithelial cells were uniform in size and shape and morphologically similar to the cells described lining biliary cysts. Mitotic figures were very rarely detected. The lumens of the microcavities were usually empty but occasionally contained granular proteinaceous material. Islands of hepatocytes were commonly entrapped in the

neoplastic tissue. In four of the 14 cases of biliary cystadenomas available for histologic evaluation, several of these cavities and the surrounding biliary cysts were filled with an abundant purulent exudate. Gram negative bacteria were scattered among the necrotic neutrophils in two of these four cases.

The main histologic feature of the 17 malignant tumours of the biliary tree available for histological evaluation was their close association with biliary cysts. Accordingly, these tumours were classified as biliary cystadenocarcinomas. Cases in the early stages were morphologically similar to cystadenomas, but also displayed multiple intracystic neoplastic proliferations, budding from the epithelial lining of the cystic structures. These epithelial growths were formed by numerous papilliform projections supported by a thin connective stroma and lined by one to two rows of densely packed, moderately disorganised, epithelial cells (Fig. 4.4B). In the more advanced cases, these intraluminal masses completely filled the biliary cysts, and markedly compressed and infiltrated the adjacent hepatic tissue. These often coalescing neoplastic nodules were then formed by numerous papilliform projections and well-differentiated tubular structures embedded in an abundant, usually poorly vascularized, fibrous stroma (Fig. 4.4C). Small nodules of hepatic parenchyma were commonly entrapped in the neoplastic and scirrhous tissues.

The level of cellular anaplasia of the neoplastic cells composing these neoplasms was moderate to high. Their vesicular nuclei were rhomboid to ovoid, often cleaved, and displayed from two- to five-fold anisokaryosis. One to two, often obvious, acidophilic nucleoli were present in each nucleus (Fig. 4.4D). Mitotic indexes were generally low,

but reached six mitoses per high power field in one case. Monstrous and binucleated cells were occasionally detected.

The cystic structures associated with these neoplasms were often filled either with a homogenous proteinaceous fluid, occasionally containing cholesterol clefts, or with a coarsely granular acidophilic material. Haemorrhages and fibrin were also observed. Aggregates of haemosiderin-laden macrophages were frequently present in the fibrous tissue. Osseous metaplasia, and extensive necrosis of the neoplastic tissue were seen in six and 12 of the 17 cases reviewed, respectively.

Metastases were detected in eight of the 17 cases for which tissue was available for histologic examination. Metastatic growths were seen on the various abdominal serosa in six cases, in abdominal lymph nodes in four cases, and in the lungs of four cases. The cellular organisation of these neoplastic implants on the serosa and in pulmonary parenchyma was similar to the primary tumours (Fig. 4.4E, 4.4F). The lymph node involvement was usually characterized by the presence of numerous well-differentiated tubular structures in the subcapsular sinuses (Fig. 4.4G). Histological features of the neoplastic cells of these metastases were also similar to those of the hepatic masses. Osseous metaplasia, and necrosis were observed in six and seven cases respectively. Histological details of biliary cystadenocarcinomas are presented in Appendix IV.

### 4.1.3 Renal tubular neoplasms

*Clinical and macroscopic findings*

Renal tubular neoplasms were slow growing masses uncommonly associated with clinical signs, all but three of 38 cases being described as incidental postmortem findings. Emaciation was observed in two ferrets with large renal neoplasms. In one of these animals, the renal tumour was diagnosed by palpation and radiographic examination eight months before the appearance of the first clinical signs.

The renal tubular neoplasms were characterized by well demarcated spherical masses which usually protruded from the renal surface (Fig. 4.5A). Up to five tumours were detected per animal (mean ● SE = 1.8 ± 0.2 tumours per affected ferret), and the size of these neoplastic growths varied from pinpoint foci of <1 mm in diameter to large tumours replacing most of the kidney (up to 22 X 16 mm). Tumours were seen in both kidneys in at least seven ferrets. On cut section, the neoplastic nodules were composed of a homogenous firm tissue, white to yellow. Central areas of necrosis and haemorrhage were present in the larger tumours (Fig. 4.5B).

Lead was not detected (< 5 ppm) in any of the four frozen kidneys submitted for toxicology.

*Histological features*

Microscopically the renal tubular neoplasms were composed of densely packed highly cellular nests separated by a thin to moderately abundant fibrous stroma. These nests of neoplastic cells were usually infiltrating the adjacent renal parenchyma (Fig. 4.5C). Remains of glomeruli, signs of the expansive nature of these tumours, were

frequently encountered in the neoplastic tissue. Larger tumours were often partially encapsulated by the compacted regional interstitial connective tissue. Of the 36 cases microscopically examined, 21 had a predominantly tubular pattern, 10 a solid organization, and five were formed by similar proportions of tubular units and solid sheets of cells. The neoplastic cells shared some morphologic similarities with renal tubular cells, and were arranged in a disorganized fashion in densely packed nests or rudimentary tubular structures.

Two different types of neoplastic cells were observed. The most common type (present in 29 of the 36 cases) was small and rhomboid, and possessed a scant, usually slightly basophilic, cytoplasm with poorly defined borders. The second type of neoplastic cell (present in 10 cases) had a more abundant finely granular acidophilic cytoplasm with poorly defined borders. The level of cellular anaplasia was, in most of the cases, low to moderate. The nuclei of both types of neoplastic cells were round to ovoid, finely vesicular, and displayed from one- to five-fold anisokaryosis. The chromatin was usually coarsely granular, and the nucleoli were small and acidophilic. Except for one case, the mitotic index was relatively low (Fig. 4.5D).

A common feature was the presence of osseous metaplasia in the center of the neoplastic masses (29 of 36 cases), characterized by the presence of networks of osseous trabeculae. These trabeculae were formed of partially mineralized osteoid matrix surrounded by well differentiated osteoblastic/osteoclastic-like cells. Bone marrow was occasionally present in this osseous tissue. The tumours were frequently associated with a scirrhous reaction (in 28 of the 36 cases), and central necrosis was present in nine cases.

Metastases were observed in only one case. This ferret had multiple nodules in the kidneys, on the abdominal serosa and in the liver. The hepatic and serosal nodules were well-delimited, were markedly compressing the adjacent parenchyma, and were composed of a dense cellular population similar to the primary renal tumours (Fig. 4.5E). Histological details of renal neoplasms are summarized in Appendix IV.

### 4.1.4 Sweat gland neoplasms

*Clinical and macroscopic findings*

Necropsy files from a total of 36 cases with neoplasms of the sweat gland were reviewed. Most of these cases were on the tail (Table 4.1). Sweat gland neoplasms, specially when affecting the tail, were usually nonaggressive and very slow growing neoplasms, seldom associated with clinical disease.

**Table 4.1** Location of neoplasms of the sweat gland in black-footed ferrets (n = 36).

| Location | Number of cases | Number of cases that metastasized |
|---|---|---|
| Tail | 24 | 1 |
| Forelimb | 1 | 1 |
| Head | 1 | 0 |
| Back | 1 | 0 |
| Abdomen | 1 | 0 |
| Vulva | 1 | 0 |
| Unspecified | 7 | 0 |
| **Total** | **36** | **2** |

Tumours of the sweat gland were described as small, often multiple, and rarely ulcerated (only five cases), subcutaneous masses. These masses were generally well-delimitated, and usually composed of several small cystic structures filled with a thick viscous to pasty grey/black material. Metastases were grossly visible in only one of the 36 cases reviewed. This animal developed a very large mass in the neck eight months after the surgical excision of a well-circumscribed subcutaneous mass from the forearm. This cervical mass was formed of a firm homogenous tan parenchyma, and was infiltrating the adjacent muscles (Fig. 4.7A and 4.7B).

*Histological features*

A total of 26 neoplasms of the sweat gland, 12 adenomas and 14 adenocarcinomas, were available for histological examination. Twenty were found on the tail, one on the forearm, one on the back, and one on the head. Exact locations of the other three tumours were not available. These neoplasms were composed of irregular, usually well-circumscribed, often cystic, generally non-encapsulated dermal masses (Fig. 4.6A). The tumours from the forearm and the back were not as well-circumscribed, and looked more aggressive than the neoplasms affecting the tail. The cellular organisation of the neoplastic masses was highly variable. Some were characterized by markedly dilated apocrine glands, filled with a homogenous proteinaceous secretion, and lined by several disorganised rows of densely packed relatively anaplastic epithelial cells. However, neoplastic nodules were generally formed by a marked intraluminal proliferation of the glandular epithelium. This hyperplastic epithelium either formed papilliform projections or well-differentiated tubular structures supported by a thin connective tissue stroma (Fig.

4.6B). These projections and tubules were lined by two to several rows of cuboidal to low cylindrical relatively well-differentiated apocrine cells. The lumens of the neoplastic tubules often contained necrotic debris and occasional neutrophils. Some cases, where this glandular differentiation was not as obvious, were formed by coalescing nodules of densely packed, markedly anaplastic, and disorganized epithelial cells (Fig. 4.7C).

The neoplastic apocrine cells were moderately to markedly anaplastic, were frequently densely packed, displayed lack of nuclear polarity, and had an abundant, and densely acidophilic cytoplasm. Their nuclei were usually ovoid, had from two- to six-fold anisokaryosis, and coarsely granular chromatin. Large densely acidophilic nucleoli were observed in several cases, and mitotic figures were, in most of the cases, rare.

The surrounding dermis was usually compressed by the tumours, and frequently contained relatively large numbers of haemosiderin-laden macrophages. The dermis also contained a high density of often dilated apocrine glands in most of the cases.

Metastases were detected in two cases. The cervical mass observed with the tumour of the forearm was a regional lymph node infiltrated by numerous neoplastic tubular and papilliform structures. This neoplastic infiltrate was associated with an extensive scirrhous reaction, and with large areas of necrosis. Small nodules composed of tubular structures were also occasionally detected in the pulmonary parenchyma. Small intravascular aggregates of neoplastic epithelial cells were occasionally seen in the lung of a ferret with a sweat gland adenocarcinoma on the tail. Interestingly, the two cases that metastasized displayed the two highest mitotic indexes (4 and 5). Histological details of sweat gland neoplasms are summarized in Appendix IV.

### 4.1.5 Mammary gland neoplasms

*Clinical and macroscopic findings*

Postmortem reports were available for 13 cases of mammary neoplasia. These tumours were usually described as well-circumscribed subcutaneous nodules on the ventral abdomen of female ferrets. The left and the right mammary glands were affected in eight and three cases respectively. The gross appearance of these masses varied from multiple cystic structures filled with a viscous fluid to compact multilobulated variously coloured growths firmly attached to the adjacent tissue. They were described as ulcerated in three cases, and enlargement of the regional lymph nodes was noted in three animals.

*Histological features*

Based on their histomorphology, the 13 mammary neoplasms reviewed were classified as follow: four simple adenomas, seven simple adenocarcinomas (four papillary, and three tubular) and two carcinosarcomas.

The simple adenomas were well-circumscribed dermal masses composed of multiple cystic glands lined by mildly hyperplastic epithelium occasionally forming short intraluminal projections. These dilated glands were surrounded by a thick fibrous stroma compressing the adjacent dermis, and were filled with a homogenous proteinaceous fluid. Intraluminal connective tissue septa, lined by one row of epithelium, were observed in one case. The epithelial cells forming these tumours were well-differentiated cuboidal cells with a low level of anaplasia. Their nuclei were usually basal in position, homogenous in sizes and in shape, and had small poorly stained nucleoli. Mitotic figures

were extremely rare.

As for the simple adenomas, simple papillary adenocarcinomas were mainly composed of dilated mammary glands. However, in adenocarcinomas, moderate to marked intraluminal papilliform proliferation of the lining epithelium was typically present (Fig. 4.8A). The neoplastic mammary glands were partially filled by complex networks of papilliform projections supported by a thin fibrous stroma and lined by one to several rows of tall cuboidal to columnar, frequently densely packed epithelial cells.

By comparison, simple tubular adenocarcinomas were formed of numerous, often coalescent, variously sized nodules of disorganised tubular and papillary structures. These nodules were separated by an abundant fibrous stroma and markedly compressed the adjacent dermis. The neoplastic tubular structures were surrounded by a thick fibrous stroma, and were formed of several layers of disorganised, densely packed, moderately to markedly anaplastic epithelial cells frequently centred on small lumens. Inspissated secretion and necrotic cellular debris were commonly observed in the lumen. Numerous intraluminal mineralized concretions were present in two of the three cases of simple tubular adenocarcinomas. The morphology of the neoplastic cells in the simple adenocarcinomas was essentially similar to that of sweat gland adenocarcinomas described above. Cells were frequently densely packed, and had an abundant brightly acidophilic cytoplasm, with undistinguishable margins. Their commonly vesicular nuclei were generally ovoid, often cleaved or irregular in shape, and displayed from two- to four-fold anisokaryosis. Large nucleoli were commonly observed in several cases, and mitotic figures were, in most of the cases, rare. Local invasion was present in two out of

four papillary adenocarcinomas and in all three tubular adenocarcinomas. Invasion seemed more aggressive in the tubular tumours.

Carcinosarcomas were poorly circumscribed, markedly invasive tumours formed of both epithelial and sarcomatous neoplastic cellular populations. Both cases were primarily composed of neoplastic epithelial tubular structures entrapped in dense sheets of highly anaplastic sarcomatous spindle-cells. The epithelial component of these tumours was similar to that in the simple tubular adenocarcinomas. The sarcomatous component had high degree of pleomorphism. These spindle-cells were commonly densely packed, were embedded in a fine connective stroma, and had an elongated slightly basophilic cytoplasm. Their highly vesicular nuclei were usually large, fusiform, often cleaved, and showed high level of anisokaryosis. Each nucleus contained one to four large densely acidophilic nucleoli, and mitotic figures were very numerous. Enormous nuclei and bizarre mitoses were commonly observed. In one case, the sarcomatous population was frequently associated with the formation of well-differentiated osseous tissue, and some neoplastic spindle-cells speculatively seemed to have differentiated into osteoblastic cells (Fig. 4.8B).

Metastases were detected in one of the three cases of simple tubular adenocarcinomas and in one of the two carcinosarcomas. In the first case, embolic aggregates of anaplastic epithelial cells were occasionally occluding pulmonary arterioles, and numerous well-differentiated tubular structures were infiltrating the medullary and subcapsular sinuses of the inguinal lymph node. A marked scirrhous reaction was associated with this neoplastic infiltrate. A small number of well-differentiated tubular

structures were seen in the subcapsular sinuses of an abdominal lymph node in the second case. Histological details of mammary gland neoplasms are summarized in Appendix IV.

### 4.1.6 Adenocarcinoma of the apocrine gland of the anal sacs

*Clinical and macroscopic findings*

Adenocarcinomas of the apocrine gland of the anal sacs were aggressive, relatively fast growing malignancies that were commonly first reported as "infection of the scent glands." These tumours were too invasive to be completely excised by surgery, and were therefore fatal in most of the cases.

Peri-anal masses, visible externally, were reported in 13 of the 16 cases. In one other case, a 7-mm diameter firm nodule was detected next to an anal sac after dissection. Two tumours were only diagnosed by microscopic examination of the peri-anal area. These subcutaneous masses were described as peri-anal masses usually markedly infiltrating the adjacent anal sacs and muscles. Seven of the 13 masses were reported to be ulcerated, and four significantly compressed the adjacent rectum. Regional lymph nodes were described as enlarged in two cases.

*Histological features*

The micro-anatomy of the anal sacs and their associated glands in black-footed ferrets is similar to that of mink (Hadlow, 1985). A high density of sebaceous and apocrine glands surround the anal sacs.

Fourteen of the 16 cases were available for histological examination. Eleven of these were characterized by non-encapsulated, often ulcerated, irregular neoplastic

growths markedly infiltrating the surrounding fat, connective and muscular tissues (Fig. 4.9A). These tumours were formed by numerous tubular structures, cellular nests, cords, and pseudolobules embedded in an extensive scirrhous stroma. Cystic cavities, filled with degenerate cells and proteinaceous secretion, were observed in some cases. The aggregates of tumour cells were disorganized, densely packed, and highly pleomorphic epithelium (Fig. 4.9B). The cytoplasm of these cells was acidophilic, relatively abundant, with indistinct borders. Small clear vacuoles, which did not contain mucus (Periodic Acid Schiff negative), were frequently observed in the cytoplasm. Nuclei were variously sized and shaped (up to 6-fold anisokaryosis), were often enormous, reniform or indented, and were usually vesicular. One to two moderately well demarcated medium sized acidophilic nucleoli were commonly present in these nuclei. Mitotic indexes were relatively high in most of the cases (up to 14 mitoses per high power field). Invasion of blood vessels by neoplastic cells was occasionally observed. Areas of necrosis, often associated with infiltration by degenerate neutrophils, were frequently present in the neoplastic tissue. Osseous metaplasia was seen in two cases.

The three other cases were characterized by the presence of well-circumscribed, non-encapsulated masses adjacent to an anal sac. These masses, which markedly compressed adjacent tissue, were formed by numerous densely packed moderately well-differentiated tubular and acinar structures separated by a light connective tissue stroma. These structures were composed of epithelial apocrine cells which were less anaplastic than the cells described in the other 11 cases.

Metastases were detected in nine cases. Anaplastic glandular and tubular

structures were infiltrating the regional lymph nodes, and the intra-abdominal lymph nodes of five and three cases respectively. Metastases were also detected in the lung of five animals, and in the spleen of one case. These metastases were randomly distributed and were formed by either small aggregates of neoplastic cells or large partially necrotic spherical areas composed of tubular structures. Histological details of adenocarcinomas of the apocrine gland of the anal sacs are summarized in Appendix IV.

### 4.1.7 Preputial apocrine gland neoplasms

*Clinical and macroscopic findings*

Three of the six cases of preputial gland tumours were symptomatic, and were described as a markedly enlarged and ulcerated preputial gland associated with haematuria.

*Histological features*

The preputial gland surrounds the preputial orifice of male black-footed ferrets. It is composed of an inner ring of sebaceous glands surrounded by an external ring of densely packed apocrine glands. Neoplastic changes were seen in the apocrine component of the gland. Tissues from six cases were examined. Three cases were classified as adenomas and three as adenocarcinomas. Microscopically, these tumours resembled the sweat gland neoplasms described above (Fig. 4.10). Scirrhous stroma was present in all three adenocarcinomas of the preputial apocrine glands, and osseous metaplasia was observed in one case.

A nodule composed of numerous glandular and tubular structures embedded in a

moderate amount of fibrous tissue was observed in the omentum of one animal with a preputial gland adenocarcinoma. Histological details of adenocarcinomas of the preputial apocrine gland are presented in Appendix IV.

### 4.1.8 Adenoma of the sebaceous gland and basal cell tumour

*Adenoma of the sebaceous gland*

Five cases of sebaceous gland neoplasia, all benign, were seen in the study group. These adenomas were described as mildly raised ulcerated cutaneous masses covered by a dry exudate. They were located on the tail (2), the foot pad (1), the back (1), and the thigh (1). Histologically, these adenomas were characterized by non-encapsulated, markedly ulcerated, masses in the superficial dermis composed of well-differentiated but disorganised sebaceous glandular structures. The general architecture of these glands was relatively preserved, aggregates of large markedly vacuolated sebaceous epithelial cells being surrounded by one to two layers of small homogenous basophilic cells. Mitotic figures were rare. Squamous metaplasia and pearls of keratin were frequently observed in the neoplastic tissue of one case. The ulcerated surface of these tumours was covered by a thick fibrinous exudate containing degenerate neutrophils, bacterial colonies and sections of hair (Fig 4.11).

*Basal cell tumours*

Two basal cell tumours, one on the ear, and one of the flank, were surgically removed from the same animal. Gross descriptions of these neoplasms were not available. The basal cell tumour from the ear was composed of relatively well-

circumscribed nodules separated by a moderately abundant fibrous stroma, and formed by densely arranged small and homogenous basophilic epithelial cells. Numerous ribbons of small basal cells embedded in an abundant connective stroma characterized the tumour from the flank.

Histological details of the adenomas of the sebaceous gland and basal cell tumours are summarized in Appendix IV.

### 4.1.9 Epidermal cyst

Epidermal cysts, described as small cystic masses filled with grey soft material, were observed on the ventral neck, and on the side of one animal each. Microscopically, these cysts moderately compressed the adjacent dermis, and were lined by a thin regular squamous epithelium. They were filled with a moderate amount of lamellar keratin, usually disposed in a concentric arrangement.

### 4.1.10 Squamous epithelial neoplasms

*Squamous cell carcinoma*

A total of 10 cases of squamous cell carcinoma were reported, seven affecting the oral cavity, two originating from the anal sacs, and one observed on the tail.

The oral squamous cell carcinomas were characterized by markedly invasive and destructive masses on the mandible in three cases, and the maxilla in four. The first clinical sign described was unilateral "swelling" of the jaw or facial bones. Time between detection of the clinical signs and death varied between three and 10 weeks.

These masses heavily infiltrated and almost completely replaced the affected bone, and were associated with gingival proliferation, and with the loss of several teeth. In most of the cases, these neoplasms were also infiltrating the surrounding osseous, muscular and connective tissues (Fig. 4.12A).

Five of the seven cases of oral squamous cell carcinoma were available for histology. Microscopically these tumours were characterized by an extensive infiltrate of numerous variously sized, often coalescent, nests of pleomorphic epithelial cells. These cellular nests were occasionally in continuity with the oral mucosa, were separated by an abundant fibrous stroma, and were composed of densely packed large epithelial cells with abundant acidophilic and frequently vacuolated cytoplasm (Fig. 4.12B). These cells possessed large, highly pleomorphic, usually vesicular, and frequently indented nuclei (up to five-fold anisokaryosis). The nucleoli (one or two per nuclei) were usually large and markedly acidophilic. Mitotic indexes varied from two to three mitoses per high power field. Desmosome-like junctions were occasionally observed between the neoplastic cells, and keratinisation of individual cells was occasionally present in the center of the cellular nests. These infiltrative neoplasms were associated with induction of fibrous stroma, and with a moderate to severe inflammatory reaction characterized by a diffuse infiltration of the fibrous stroma and of the neoplastic nests by necrotic neutrophils and mononuclear cells. Remnants of osseous tissue surrounded by osteoclastic cells were frequently observed in the neoplastic tissue. Focal pulmonary metastases were detected in one case.

The squamous cell carcinoma observed on the tail was described as a small

papilliform growth. This neoplasm resembled the oral tumours histologically.

Both squamous cell carcinomas of the anal sac were described as peri-anal masses (ulcerated in one case) centered on one anal sac. These masses were composed of numerous markedly anaplastic cellular cords and nests surrounded by an abundant fibrous stroma. The neoplastic cells had an abundant acidophilic cytoplasm and frequently formed palisades. Their nuclei showed a high degree of anisokaryosis, and were often enormous, indented, and vesicular. One to two large acidophilic nucleoli were easily detected per cell. Mitoses were commonly seen. Continuity between these neoplasms and the squamous lining of the anal sac was obvious in both cases.

Histological details of the epidermal cysts and squamous cell carcinomas are presented in Appendix IV.

4.1.11 Other neoplasms

*Malignant ocular melanoma*

A malignant ocular melanoma was diagnosed in one ferret with a history of uveitis. This intra-ocular mass was centred on the ciliary body, and completely filled the iridocorneal angle. The mass was formed of several nodules infiltrating the adjacent sclera and cornea. These nodules were composed of a disorganized and dense network of large polygonal- to spindle-shaped cells separated by a discrete fibrous stroma. Cytoplasm was abundant, acidophilic, and often filled with granules of melanin. Their nuclei displayed marked pleomorphism (up to five-fold anisokaryosis), were usually vesicular, and possessed one to two obvious basophilic nucleoli. Mitoses were rare.

*Vascular neoplasms*

Two haemangiomas and one haemangiosarcoma were diagnosed. Both haemangiomas were small masses submitted as surgical biopsies (locations unknown), and were formed by variously sized blood filled cavities lined by a well-differentiated endothelium, and supported by a moderately abundant connective tissue stroma.

The haemangiosarcoma was described as a subcutaneous nodule on the neck and was composed of solid sheets of spindle cells. These elongated cells often formed variously sized blood-filled cavities, and had a slightly basophilic cytoplasm with indistinct borders and an elongated variously sized nucleus (up to five-fold anisokaryosis). These nuclei were often vesicular, occasionally indented and possessed one to two moderately apparent acidophilic nucleoli. Mitoses were not common. Large areas of necrosis were commonly observed in the neoplastic tissue.

*Uterine leiomyoma*

This benign neoplasm was encountered in two animals, and was described as multinodular masses affected uterine horns. These masses were essentially composed of bundles of fairly homogeneous fusiform cells interlacing in a disordered manner, frequently at a 90° angle. These cells had a relatively abundant acidophilic cytoplasm, and possessed uniform, usually central, "cigar shaped" nuclei with small discrete nucleoli. Mitoses were rarely observed.

*Fibroma*

A fibroma was diagnosed at a vaccination site in one animal. This mass was relatively well-circumscribed and was composed of dense sheets of well-differentiated

fibroblasts replacing the normal dermal architecture and infiltrating the adjacent muscle and fat.

*Spindle-cell neoplasm*

One tumour from an unknown location was classified as an undifferentiated spindle-cell neoplasm. The poor differentiation of the markedly anaplastic spindle-cells forming this mass, the lack of macroscopic description, and the absence of normal tissue in the section examined prevent the identification of the cellular origin of this neoplasm.

*Olfactory neuroblastoma*

Olfactory neuroblastomas were reported in two ferrets. One of these cases was described as a soft grey-white vascular mass filling the nasal cavity, displacing the nasal septum, and extending through the cribriform plate into the olfactory tubercule and cerebral cortex. A macroscopic description was not available for the other case.

Histologically, these tumours were characterized by the invasion of the cribriform plate and cerebral parenchyma by numerous cords and nodules of small densely packed ovoid basophilic cells, with large central areas of necrosis. These cells were often disposed in palisades and rosettes, and showed a moderate level of anisokaryosis. Their cytoplasm had undistinct borders, and their nuclei were ovoid to reniform and had a coarse chromatin. Mitotic figures were very common.

*Ganglioneuroblastoma*

A ganglioneuroblastoma was diagnosed in one ferret. This mass was not mentioned in the macroscopic examination, and the anatomic location could not be determined. Microscopically this tumour was formed of a well-circumscribed

multilobulated mass composed of loosely organised homogenous ovoid to polygonal cells with an irregular and fibrillar cytoplasm. These neoplastic cells had small ovoid to elongated dark nuclei, and were often grouped in small nests surrounded by a moderately abundant finely fibrillar connective tissue stroma. Sections of nerves were commonly observed in the fibrous tissue surrounding the tumour.

*Testicular neoplasms*

Testicular neoplasms were observed in two animals. A seminoma and an interstitial cell tumour were detected in the same testis during the postmortem examination of one animal. An interstitial cell tumour was associated with a swollen testis in another ferret.

The seminoma was characterized by a large well-circumscribed mass markedly compressing the adjacent seminiferous tubules. The neoplastic cells composing this tumour were uniform in size and shape, and occasionally were arranged in tubular structures or nodules supported by a thin, well-vascularized fibrous stroma. The cytoplasm of these cells was finely granular, and their nuclei were usually ovoid and vesicular. Enormous striking nucleoli were occasionally observed. The interstitial cell tumours were formed by moderately invasive masses composed of dense sheets of uniform finely vacuolated polygonal cells with small, often eccentric, nuclei. Large round vacuoles were commonly observed between the neoplastic cells.

*Transitional cell carcinoma*

Based on its histologic features and behaviour, one neoplasm was believed to be a transitional cell carcinoma. Multiple markedly infiltrative masses were observed in the

pelvic inlet and in the surrounding muscular, connective, and osseous tissues. Splenomegaly, diffuse lymphadenopathy, and enlarged adrenal glands were also reported.

Markedly pleomorphic epithelial neoplastic cells were massively and diffusely invading the perirenal fat, the spleen, the abdominal lymph nodes, the adrenal glands, the abdominal wall, the omentum, and the testes. Aggregates of neoplastic cells were also commonly observed in the dermis and tracheal submucosa. On one section, the neoplastic cells seemed to originate from the mucosa of the renal pelvis. The neoplastic cells were often grouped in small loosely packed cellular nests supported by a moderately abundant connective tissue stroma. These polygonal cells had an abundant cytoplasm with obvious borders, were often binucleated, and displayed a very high degree of anisokaryosis. The nuclei were vesicular, often cleaved, and commonly gigantic. Large basophilic nucleoli were common, and mitoses were very common.

*Nasal carcinoma*

Two cases of nasal carcinoma were reported. In one of these cases, the tumour was described as multiple cystic structures filled with thick clear mucoid liquid in the sinuses and the right anterior cerebrum.

One of these two cases was available for microscopical examination. The tumour was formed by fine networks of several masses composed of well-differentiated tubular and glandular structures supported by a loose connective stroma. These structures were composed of uniform cuboidal epithelial cells displaying moderately anaplastic ovoid nuclei. The cytoplasm of these cells often contained mucoid secretions, and occasional cells were bordered by poorly defined cilia. Sections of mildly compressed cerebral

tissue were occasionally seen.

*Abdominal epithelial neoplasms of undetermined origin*

Intra-abdominal neoplasms, of undetermined origin, were seen in three ferrets. A mass was present at the root of the mesentery of one animal. This mass was formed of numerous nodules of autolysed epithelial cells separated by an abundant fibrous stroma. A mass, located dorsal to the prostate, was seen in another ferret. This periprostatic mass was formed of highly anaplastic epithelial cells usually arranged in tubules supported by an abundant connective stroma. Finally, in the third case, several masses were observed in the pelvic inlet compressing the right ureter. The iliac lymph nodes of this animal were enlarged. Freezing artefact prevented characterization of the epithelial cells composing this neoplastic growth.

Histological details of these neoplasms are summarized in Appendix IV.

**Figure 4.1A** Intrahepatic biliary cysts (C) in a 6-yr-old male (SB#: 393). Bar = 1 cm.

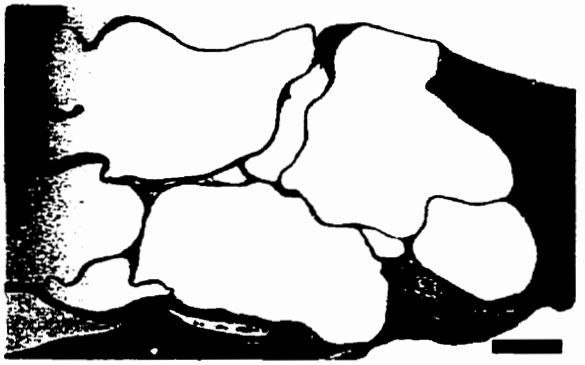


**Figure 4.1B** Histology section of biliary cysts in a 6-yr-old female (SB# 135). Cystic cavities surrounded by a thin fibrous stroma are in the liver. H&E stain. Bar = 600 $\mu$m.

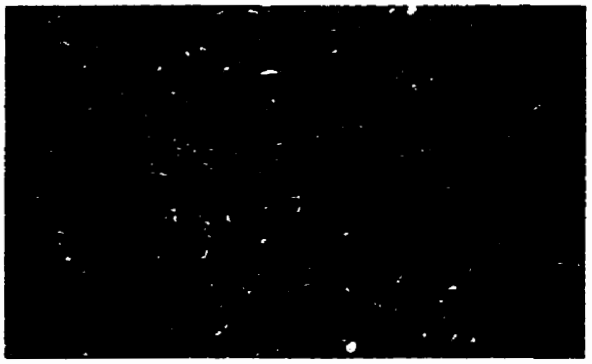


**Figure 4.2A** Foci of cholangiocellular hyperplasia in a 6-yr-old male (SB#: 110). Note the mild lymphocytic infiltration. H&E stain. Bar = 60 $\mu$m.

**Figure 4.2B** Cholangiohamartoma-like lesion in the liver of a 6-yr-old male (SB#: 188). Clusters of mildly dilated disorganised biliary ducts embedded in a fibrous stroma. H&E stain. Bar = 150 $\mu$m.

**Figure 4.2C** Cluster of poorly differentiated epithelial cells, most likely cholangiocellular precursors in a 4-yr-old male (SB#: 916). H&E stain. Bar = 20 $\mu$m.

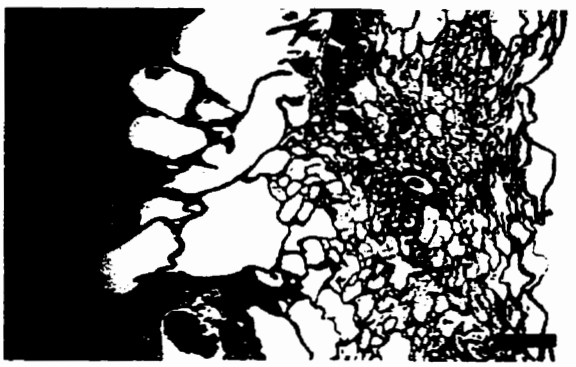


**Figure 4.3A** Biliary cystadenoma in a 6-yr-old female (SB#: 445). Complex network of ductular microcavities. H&E stain. Bar = 500 $\mu$m

**Figure 4.3B** Higher magnification of figure 4.3A showing intraluminal projections lined by a flattened epithelium. H&E stain. Bar = 50 $\mu$m.

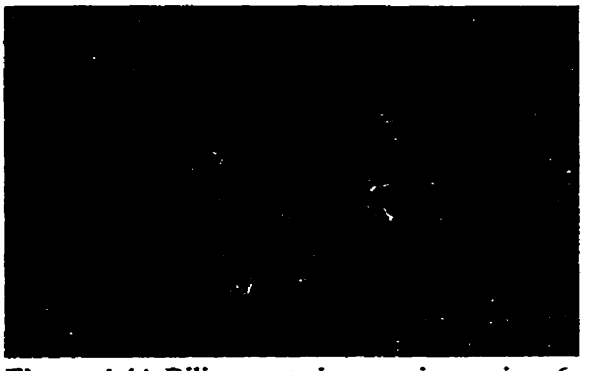


**Figure 4.4A** Biliary cystadenocarcinoma in a 6-yr-old female (SB#: 448). Large multilobulated mass. Bar = 1 cm.


**Figure 4.4B** Biliary cystadenocarcinoma in an 8-yr-old female (SB#: 31). Intracystic papilliform proliferations. C: lumen of the cyst; A: abdominal cavity. H&E stain. Bar = 250 $\mu$m.

**Figure 4.4C** Biliary cystadenocarcinoma in an 8-yr-old male (SB#: 13). Neoplastic masses completely fill the cystic cavities forming coalescent neoplastic nodules. H&E stain. Bar = 100 $\mu$m.

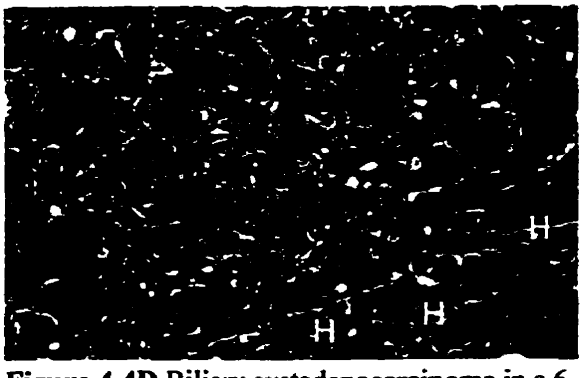



**Figure 4.4D** Biliary cystadenocarcinoma in a 6-yr-old female (SB#: 448). Markedly anaplastic cholangiolar cells infiltrating and compressing the adjacent hepatic parenchyma (H). H&E stain. Bar = 40 $\mu$m.

**Figure 4.4E** Pulmonary metastasis of biliary cystadenocarcinoma in a 4-yr-old male (SB#: 230). H&E stain. Bar = 20 $\mu$m.

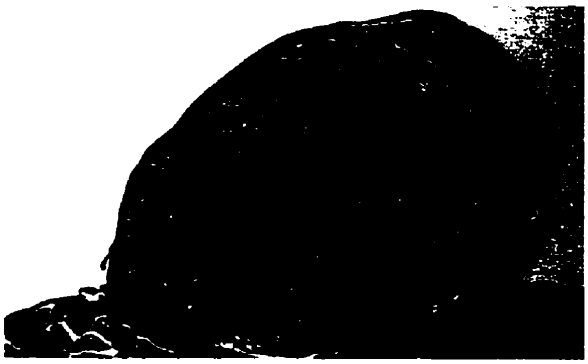


**Figure 4.4F** Metastatic nodule of biliary cystadenocarcinoma on the diaphragm of a 6-yr-old male (SB#: 110). H&E stain. Bar = 450 $\mu$m.

**Figure 4.4G** Metastasis of biliary cystadenocarcinoma in the lymph node of a 5-yr-old male (SB#: 16). H&E stain. Bar = 130 $\mu$m.

**Figure 4.5A** Multiple renal tubular neoplasms (arrowheads) in an 8-yr-old male (SB#: 86). Bar = 1 cm.

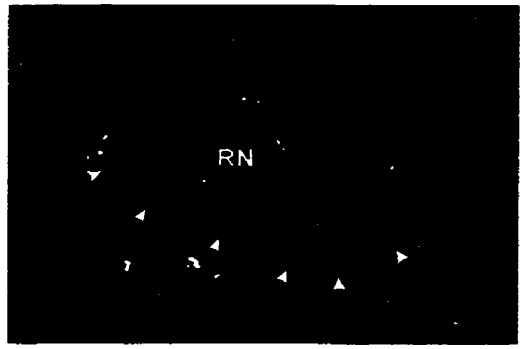



**Figure 4.5B** Large renal tubular neoplasm (RN) replacing most of the renal tissue in a 6-yr-old male (SB#: 393). Mass delimitated by arrowheads. Bar = 1 cm.

**Figure 4.5C** Two infiltrative renal tubular neoplasms in an 8-yr-old female (SB#: 40). Osseous metaplasia is present in the center of one tumour. H&E stain. Bar = 330 $\mu$m.

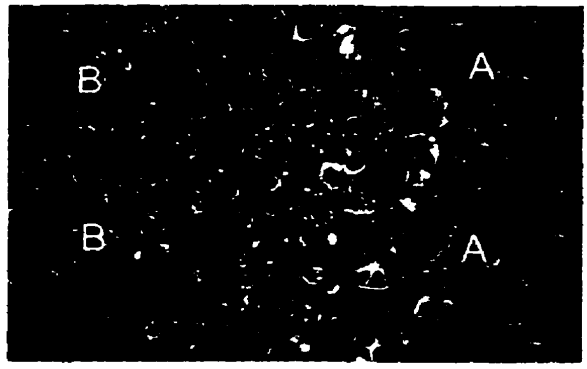



**Figure 4.5D** Renal tubular neoplasm in a 7-yr-old male (SB#: 129). Both types of neoplastic cells are present; small-basophilic cells (B), and larger acidophilic cells (A). H&E stain. Bar = 60 $\mu$m.


**Figure 4.5E** Metastases (M) of renal tubular neoplasm in the liver of a 5-yr-old male (SB#: 171). H&E stain. Bar = 500 $\mu$m.

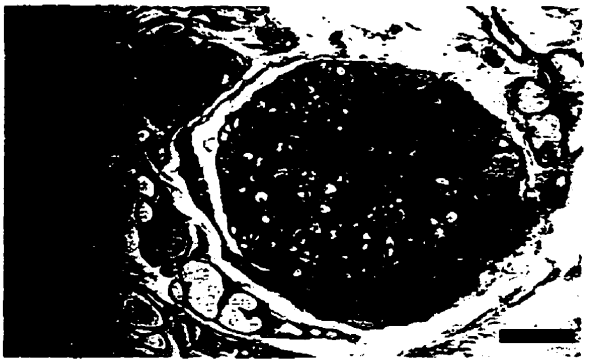


**Figure 4.6A** Sweat gland adenoma on the tail of a 8-yr-old male (SB#: 86). Slightly dilated sweat glands in the adjacent dermis (SG). H&E stain. Bar = 500 $\mu$m.

**Figure 4.6B** Higher magnification of figure 4.6A showing well differentiated glandular structures lined by homogenous cuboidal apocrine epithelial cells. H&E stain. Bar = 30 $\mu$m.

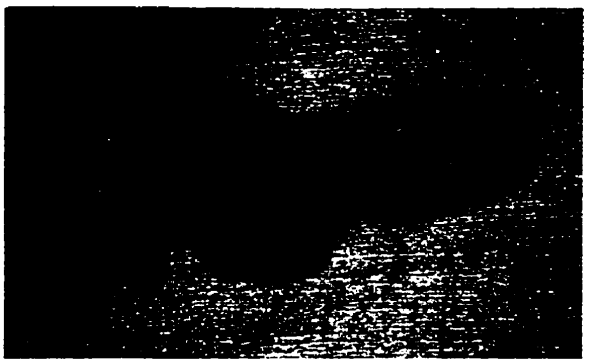


**Figure 4.7A** Sweat gland adenocarcinoma on the forelimb of a 7-yr-old female (SB#: 173). Bar = 1 cm.

**Figure 4.7B** Same animal as figure 4.7A, 8 months after the surgical excision of the primary tumour. Markedly enlarged scapular lymph node (LN) associated with extensive metastasis. Neck and head to the rigth. Bar = 1 cm.

**Figure 4.7C** Sweat gland adenocarcinoma of the tail of a 6-yr-old male (SB#: 101). Densely packed markedly anaplastic epithelial cells. H&E stain. Bar = 60 $\mu$m.

**Figure 4.8A** Simple papillary adenocarcinoma of the mammary gland in an 8-yr-old female (SB#: 40). Invasion of the sclerosed dermis (D) by cystic glands with intraluminal papilliform proliferation. H&E stain. Bar = 500 $\mu$m.


**Figure 4.8B** Carcinosarcoma of the mammary gland in a 7-yr-old female (SB#: 27). Glandular (G) and sarcomatous (S) neoplastic populations are present. Osseous metaplasia (arrowheads) is observed. H&E stain. Bar = 90 $\mu$m.

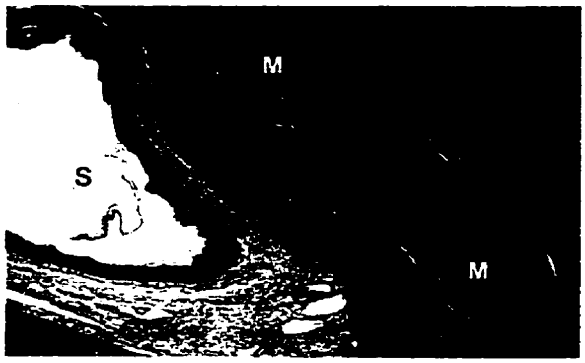



**Figure 4.9A** Adenocarcinoma of the apocrine gland of the anal sacs in a 6-yr-old male (SB#: 188). The invasive glandular mass (M) compresses the adjacent anal sac (S). H&E stain. Bar = 400 $\mu$m.

**Figure 4.9B** Higher magnification of figure 4.9A showing the markedly anaplastic epithelial cells forming disorganized palisades. Note the marked anisokaryosis and the mitotic figures. H&E stain. Bar = 50 $\mu$m.

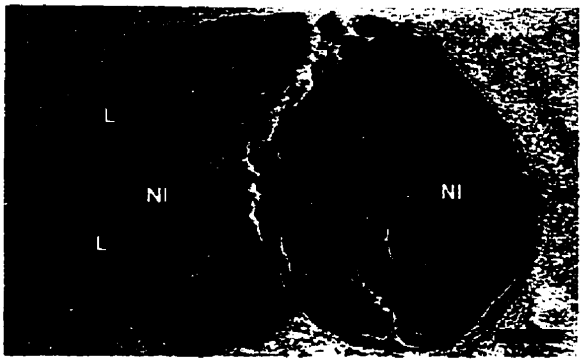



**Figure 4.9C** Regional lymph node of SB# 188. The lymphoid tissue (L) is almost completely replaced by the neoplastic infiltrate (NI). H&E stain. Bar = 500 $\mu$m.

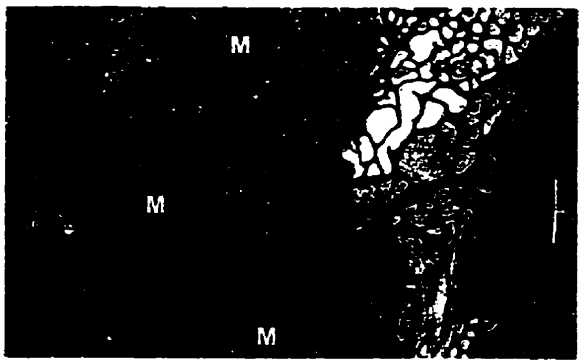



**Figure 4.10** Adenocarcinoma of the apocrine preputial gland in a 7-yr-old male (SB#: 77). Large neoplastic mass (M) infiltrating the preputial gland (PG). H&E stain. Bar = 500 $\mu$m.

**Figure 4.11** Sebaceous adenoma in an 8-yr-old male (SB#: 85). The well-circumscribed mass is ulcerated. H&E stain. Bar = 500 $\mu$m.

**Figure 4.12A** Oral squamous cell carcinoma in a 4-yr-old male (SB#: 278). Large mandibular mass (arrowheads). Note the marked displacement of the tongue.

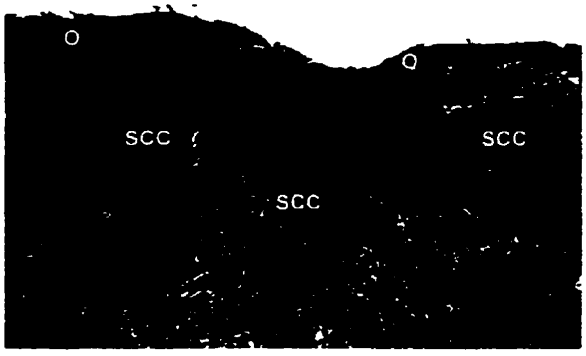



**Figure 4.12B** Histologic section of the mandibular mass of SB#: 278. The continuity between the neoplastic tissue (SCC) and the oral mucosa (O) is obvious. H&E stain.
Bar = 150 $\mu$m.

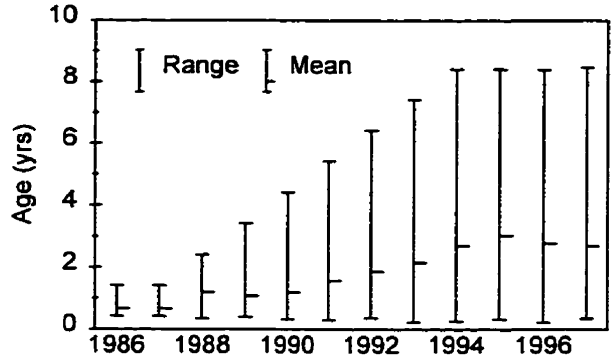
## 4.2 Epidemiology of neoplasia in black-footed ferrets

### 4.2.1 Characteristics of the study population and study group

*Study population*

The study population (733 males, 690 females, and 449 ferrets of unknown gender, total 1872 animals - Table 3.1, page 26) was used to determine the estimates of annual incidence. Mean ages for this population progressively increased during the first eight years of the breeding program (Fig. 4.13). Mean ages stabilized and animals appeared to have attained the maximum life-span in 1994.

**Figure 4.13** Age distribution of the study population of black-footed ferrets over the period 1986 to 1997. Wild caught ferrets of unknown age not included.




*Study group*

The study group (103 males and 81 females; total 184 - Appendix VII) is composed of all the animals for which sufficient information about postmortem examinations was available. Comparisons between males and females for age and inbreeding coefficient are summarized in Table 4.2 (page 75). No differences were

observed between males and females as far as age distribution, inbreeding coefficient, and time spent in all but one institution. Males spent significantly more time in institutions other than the seven breeding centres than females ($p = 0.011$, Wilcoxon rank sum test).

### 4.2.2 Annual rates of neoplasia in the study population

Crude annual rates (CAR) and life span adjusted annual rates (LSAAR) per 100,000 animals for the most common type of neoplasms are presented in Tables 4.3 and 4.4 (pages 77 and 78). Rates for the four last years of the study, and for the entire period of the study were also calculated.

### 4.2.3 Prevalence of neoplasia at death in the study group

A total of 185 tumours, classified into 28 phenotypes, was detected in 102 of the 184 ferrets in the study group. Prevalences for these neoplasms and their relative frequencies are presented in Table 4.5 (page 78). The three most common tumours, the renal tubular neoplasms, the biliary cystadenomas/cystadenocarcinomas, and the tumours of the sweat gland, were each seen in approximately 20% of the animals. A comparison of the prevalences of the most common neoplasms in males and females is presented in Table 4.6 (page 80). Mammary gland neoplasms were only encountered in females, and tumours of the sweat gland, and of the apocrine gland of the anal sacs were significatively more frequent in males.

The prevalence at death of the different conditions was greatly influenced by age. The strong relationship between age and prevalence is presented in Figure 4.14 (page 81).

### 4.2.4 Number of tumours per animal

The numbers of neoplasms, and of invasive neoplasms, per animal are given in Table 4.7 (page 83). Multiple neoplasms were very common in black-footed ferrets, 50% of the animals with neoplasia being affected by more than one tumour, and up to six neoplasms being observed in a single animal. The number of neoplasms per animal was significantly correlated with the age of the animal at death (Fig. 4.15, page 83).

### 4.2.5 Evaluation of Mendelian inheritance

The relationship between the tumour status of the parents for each event of interest and the frequency of these events in their offsprings are presented in Table 4.8 (page 84). A significant association between the number of parents affected and the status of their offspring could not be demonstrated for any event of interest examined. Consequently, the disease status of the parents does not seem to affect the likelihood that their first generation descendants will be affected by the same condition.

Table 4.9 (page 84) explores the relationship between the tumour status of the parents and the age at onset for each event of interest in their offspring. Significant differences were not observed for any of the syndromes.

### 4.2.6 Associations between neoplasia and environmental and host factors

The results obtained from the logistic regression analysis for each event of interest are presented in Table 4.10 (page 85). Only the variables that improved the fitness of

each model are presented.

"Age" was a determinant for all the conditions examined. The most dramatic effect was observed for the biliary cysts where each year of life was associated with an increase in odds of disease of approximately five times. The odds of adenocarcinoma of the apocrine gland of the anal sacs, a sweat gland neoplasm, a renal tubular neoplasm, a biliary cystic neoplasm, or a tumour of the mammary glands also increased with aging (approximately two to three times per year of life). Males were more likely to be affected by sweat gland neoplasms (approximately four times), and by adenocarcinomas of the apocrine gland of the anal sacs (approximately 16 times), than females. An increase in the time spent at the Toronto Zoo was associated with an increase in the odds of being diagnosed with a sweat gland neoplasm, or a renal tubular neoplasm. In contrast, an increase in the time spent at the National Black-Footed Ferret Conservation Center was associated with a decrease of the odds of adenocarcinoma of the apocrine gland of the anal sacs. The date of birth had an effect on the chance to be affected by biliary cysts. The only other variable that had a significant effect on the presence of the conditions examined was the percentage of first generation relatives affected by an adenocarcinoma of the anal sacs. The chance to be affected by this tumour actually decreased when this percentage increased.

### 4.2.7 Clinical disease and deaths attributed to biliary cysts and neoplasia

Based on postmortem results and clinical files, neoplasia was recognized as the cause of death (natural or by euthanasia) in 62 animals, which represents 33.7% of the

ferrets from the study group. The age distribution of these cases for males and females is presented in Figure 4.16 (page 87). We can see that with the exception of two 3-yr-old and three 4-yr-old males, all the ferrets that died due to neoplasia were older than 5 years of age.

"Symptomatic ratios" and "fatality ratios" for the most common conditions were calculated (Table 4.11, page 87). Biliary cystadenocarcinoma, adenocarcinoma of the apocrine gland of the anal sacs, mammary gland neoplasm, and squamous cell carcinoma were associated with a high mortality.

### 4.2.8 Comparative epidemiology of neoplasia in black-footed ferrets

The study population reached "maturity" in 1994 (Fig. 4.13, page 70). Consequently, the crude annual rate (CAR) of neoplasia recorded during the four last years of the study represents the best estimate of the incidence of neoplastic diseases for this population. Compared to other species, captive black-footed ferrets have a very high crude annual rate of neoplasia (Table 4.12, page 88). When adjusted to human life span (LSAAR) this rate remains relatively high, but is in the same range as the Canadian and American human populations, and domestic animals (Table 4.13, page 89).

The prevalence at death of neoplasia in black-footed ferrets is compared with rates reported for other species in Table 4.14 (page 90). The prevalence at death of neoplasia in black-footed ferrets is far higher than information published for domestic ferrets, mink, and mammals held in zoological collections, but is in the same range as that described in Sprague-Dawley rats, and people (Table 4.14, page 90).

The LSAAR and prevalence at death for renal neoplasia in black-footed ferrets is extremely high when compared to other species (Table 4.15, page 91). The LSAAR for mammary tumours in black-footed ferrets is similar to what has been described for dogs and people, and the prevalence is in the same range as that of laboratory rats (Table 4.16, page 92).

**Table 4.2** Age and inbreeding coefficient of male and female black-footed ferrets of the study group.

| Variable | Mean ± STD | | p-value[c] |
|---|---|---|---|
| | Female | Male | |
| Age (years)[a] | 5.78 ± 2.20 | 5.50 ● 1.99 | 0.385 |
| Inbreeding coefficient[b] | 0.10 ± 0.06 | 0.09 ● 0.04 | 0.347 |

[a] Female: n = 78; male: n = 100.
[b] Female: n = 38; male: n = 62.
[c] Two-sample t-test.

**Table 4.3** Crude annual rates of neoplasms in the study population (n = 184) of black-footed ferrets, 1988 - 1997 inclusive. No neoplasms were diagnosed before 1988.

| | Biliary cysts | Deaths attributed to neoplasia | Invasive neoplasms | All neoplasms | All epithelial neoplasms | All apocrine neoplasms | Biliary neoplasms | Renal tubular neoplasms | Sweat gland neoplasms | Apocrine neoplasms - anal sacs | Mammary neoplasms | Preputial neoplasms | Squamous cell carcinoma | Sebaceous adenoma |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Number of cases with the event of interest | | | | | | | | | | | | | |
| | 115 | 53 | 87 | 95 | 81 | 47 | 35 | 34 | 32 | 15 | 13 | 6 | 8 | 5 |
| Years | Crude annual rates per 100,000 animals | | | | | | | | | | | | | |
| 1988 | 2581 | 2581 | 2581 | 2581 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1989 | 1167 | 1167 | 1167 | 1167 | 1167 | 1167 | 0 | 0 | 1167 | 0 | 0 | 0 | 1167 | 1167 |
| 1990 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1991 | 0 | 0 | 0 | 431 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1992 | 919 | 919 | 919 | 919 | 919 | 612 | 919 | 306 | 306 | 0 | 0 | 306 | 0 | 0 |
| 1993 | 1049 | 787 | 1050 | 1049 | 787 | 262 | 262 | 525 | 262 | 0 | 262 | 0 | 262 | 0 |
| 1994 | 2752 | 1835 | 2752 | 2752 | 1835 | 1147 | 229 | 1147 | 459 | 459 | 688 | 0 | 917 | 0 |
| 1995 | 5262 | 1535 | 3508 | 3947 | 3508 | 1316 | 1535 | 1535 | 1096 | 439 | 439 | 0 | 0 | 219 |
| 1996 | 8257 | 3342 | 5898 | 6488 | 5898 | 3539 | 2949 | 1573 | 2163 | 1573 | 590 | 983 | 197 | 590 |
| 1997 | 5533 | 2569 | 3952 | 4347 | 3952 | 2767 | 1581 | 2174 | 2174 | 593 | 790 | 0 | 198 | 0 |
| **4 yrs [a]** | **5559** | **2360** | **4091** | **4458** | **3881** | **2255** | **1626** | **1626** | **1521** | **787** | **629** | **262** | **315** | **210** |
| **12 yrs [b]** | **3650** | **1682** | **2762** | **3015** | **2571** | **1492** | **1111** | **1079** | **1016** | **476** | **413** | **190** | **254** | **159** |

[a] CAR for the four last years of the study (1994-1997).
[b] CAR for the 12 years of the study.

**Table 4.4** Life span adjusted annual rate (LSAAR) of neoplasms in the study population (n = 184) of black-footed ferrets, 1988 - 1997 inclusive. No neoplasms were diagnosed before 1988.

| | Biliary cysts | Deaths attributed to neoplasia | Invasive neoplasms | All neoplasms | All epithelial neoplasms | All apocrine neoplasms | Biliary neoplasms | Renal tubular neoplasms | Sweat gland neoplasms | Apocrine neoplasms - anal sacs | Mammary neoplasms | Preputial neoplasms | Squamous cell carcinoma | Sebaceous adenoma |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Number of cases with the event of interest | | | | | | | | | | | | | |
| | 115 | 53 | 87 | 95 | 81 | 47 | 35 | 34 | 32 | 15 | 13 | 6 | 8 | 5 |
| Years | Life span adjusted annual rates per 100,000 animals | | | | | | | | | | | | | |
| 1988 | 258 | 258 | 258 | 258 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1989 | 117 | 117 | 117 | 117 | 117 | 117 | 0 | 0 | 117 | 0 | 0 | 0 | 117 | 117 |
| 1990 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1991 | 0 | 0 | 0 | 43 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1992 | 92 | 92 | 92 | 92 | 92 | 61 | 92 | 31 | 31 | 0 | 0 | 31 | 0 | 0 |
| 1993 | 105 | 79 | 105 | 105 | 79 | 26 | 26 | 53 | 26 | 0 | 26 | 0 | 26 | 0 |
| 1994 | 275 | 184 | 275 | 275 | 184 | 115 | 23 | 115 | 46 | 46 | 69 | 0 | 92 | 0 |
| 1995 | 526 | 154 | 351 | 395 | 351 | 132 | 154 | 154 | 110 | 44 | 44 | 0 | 0 | 22 |
| 1996 | 826 | 334 | 590 | 649 | 590 | 354 | 295 | 157 | 216 | 157 | 59 | 98 | 20 | 59 |
| 1997 | 553 | 257 | 395 | 435 | 395 | 277 | 158 | 217 | 217 | 59 | 79 | 0 | 200 | 0 |
| **4 yrs [a]** | **556** | **236** | **409** | **446** | **388** | **226** | **163** | **163** | **152** | **79** | **63** | **26** | **32** | **21** |
| **12 yrs [b]** | **365** | **168** | **276** | **302** | **257** | **149** | **111** | **108** | **102** | **48** | **41** | **19** | **25** | **16** |

[a] LSCAR for the four last years of the study (1994-1997).
[b] LSCAR for the 12 years of the study (1986-1997)

**Table 4.5** Prevalence of biliary cysts and neoplasms at death and relative frequencies of neoplasms for black-footed ferrets in the study group.

| Conditions | No. of cases | Prevalence [a] (%) | Frequencies [b] (%) |
|---|---|---|---|
| Biliary cyst | 121 | 65.76 | - |
| Total neoplasms | 185 | 55.43 | 100 |
| Total epithelial neoplasms | 171 | 47.28 | 92.43 |
| Renal tubular neoplasm | 38 | 20.65 | 20.54 |
| Total biliary neoplasm | 37 | 20.11 | 20 |
| Cystadenoma | 14 | 7.61 | 7.57 |
| Cystadenocarcinoma | 23 | 12.5 | 12.43 |
| Total apocrine neoplasms | 71 | 28.26 | 38.38 |
| Total sweat gland neoplasms | 36 | 19.57 | 19.46 |
| Adenoma | 13 | 7.07 | 7.03 |
| Adenocarcinoma | 23 | 12.5 | 12.43 |
| Total mammary gland neoplasms | 13 | 7.07 | 7.03 |
| Adenoma | 3 | 1.63 | 1.62 |
| Adenocarcinoma | 10 | 5.43 | 5.41 |
| Adenocarcinoma - apocrine gland, anal sacs | 16 | 8.7 | 8.65 |
| Total preputial gland neoplasms | 6 | 3.26 | 3.24 |
| Adenoma | 3 | 1.63 | 1.62 |
| Adenocarcinoma | 3 | 1.63 | 1.62 |
| Sebaceous gland adenoma | 5 | 2.72 | 2.7 |
| Total squamous cell carcinomas | 8 | 4.35 | 4.32 |
| Oral squamous cell carcinoma | 7 | 3.8 | 3.78 |
| Cutaneous squamous cell carcinoma | 1 | 0.54 | 0.54 |
| Carcinoma of the anal sac | 2 | 1.09 | 1.08 |
| Epidermal cyst | 2 | 1.09 | 1.08 |
| Basal cell tumour | 2 | 0.54 | 1.08 |
| Nasal carcinoma | 2 | 1.09 | 1.08 |
| Transitional cell carcinoma | 1 | 0.54 | 0.54 |
| Undetermined epithelial neoplasm | 3 | 1.63 | 1.62 |

**Table 4.5 cont.**

| Conditions | No. of cases | Prevalence [a] (%) | Frequencies [b] (%) |
|---|---|---|---|
| Non-epithelial neoplasms | 14 | 7.07 | 7.57 |
| Total vascular neoplasms | 3 | 1.63 | 1.62 |
| Haemangioma | 2 | 1.09 | 1.08 |
| Haemangiosarcoma | 1 | 0.54 | 0.54 |
| Fibroma | 1 | 0.54 | 0.54 |
| Uterine leiomyoma | 2 | 1.09 | 1.08 |
| Spindle cell tumour of soft tissue | 1 | 0.54 | 0.54 |
| Ocular malignant melanoma | 1 | 0.54 | 0.54 |
| Ganglioneuroblastoma | 1 | 0.54 | 0.54 |
| Olfactory neuroblastoma | 2 | 1.09 | 1.08 |
| Seminoma | 1 | 0.54 | 0.54 |
| Interstitial cell tumour | 2 | 1.09 | 1.08 |

[a] Percentage of animals affected by neoplasia in the study group (n = 184).

[b] Relative frequency of each tumour type as a proportion of the total number of tumours (n = 185).

**Table 4.6** Prevalence at death for biliary cysts and for the most common neoplasms in male (n = 103) and female (n = 81) black-footed ferrets in the study group.

| Events of interest | Prevalence (%) | | |
|---|---|---|---|
| | Male | Female | p-value[a] |
| Biliary cyst | 66.99 | 64.2 | 0.692 |
| At least one neoplasm | 56.31 | 54.32 | 0.788 |
| At least one epithelial neoplasm | 47.57 | 46.91 | 0.966 |
| At least one apocrine epithelial neoplasm | 30.1 | 25.93 | 0.533 |
| Renal tubular neoplasm | 18.45 | 23.46 | 0.405 |
| Biliary cystadenoma/cystadenocarcinoma | 21.36 | 18.52 | 0.633 |
| Sweat gland neoplasm | 24.27 | 13.58 | 0.007[b]* |
| Adenocarcinoma - apocrine gland, anal sacs | 13.59 | 2.47 | 0.014[b]* |
| Mammary gland neoplasm | 0 | 16.05 | 0.001* |
| Squamous cell carcinoma | 3.88 | 4.94 | 0.733 |
| Preputial gland neoplasm | 5.83 | - | 0.035* |
| Sebaceous adenoma | 2.91 | 2.47 | 1 |

[a] Two-sample t-test if not otherwise specified.
[b] Logistic regression.
* Statistically significant differences.

**Figure 4.14** Age distribution of biliary cysts and of the most common neoplasms in the study group (n = 171; age was available for 98 males and 73 females).

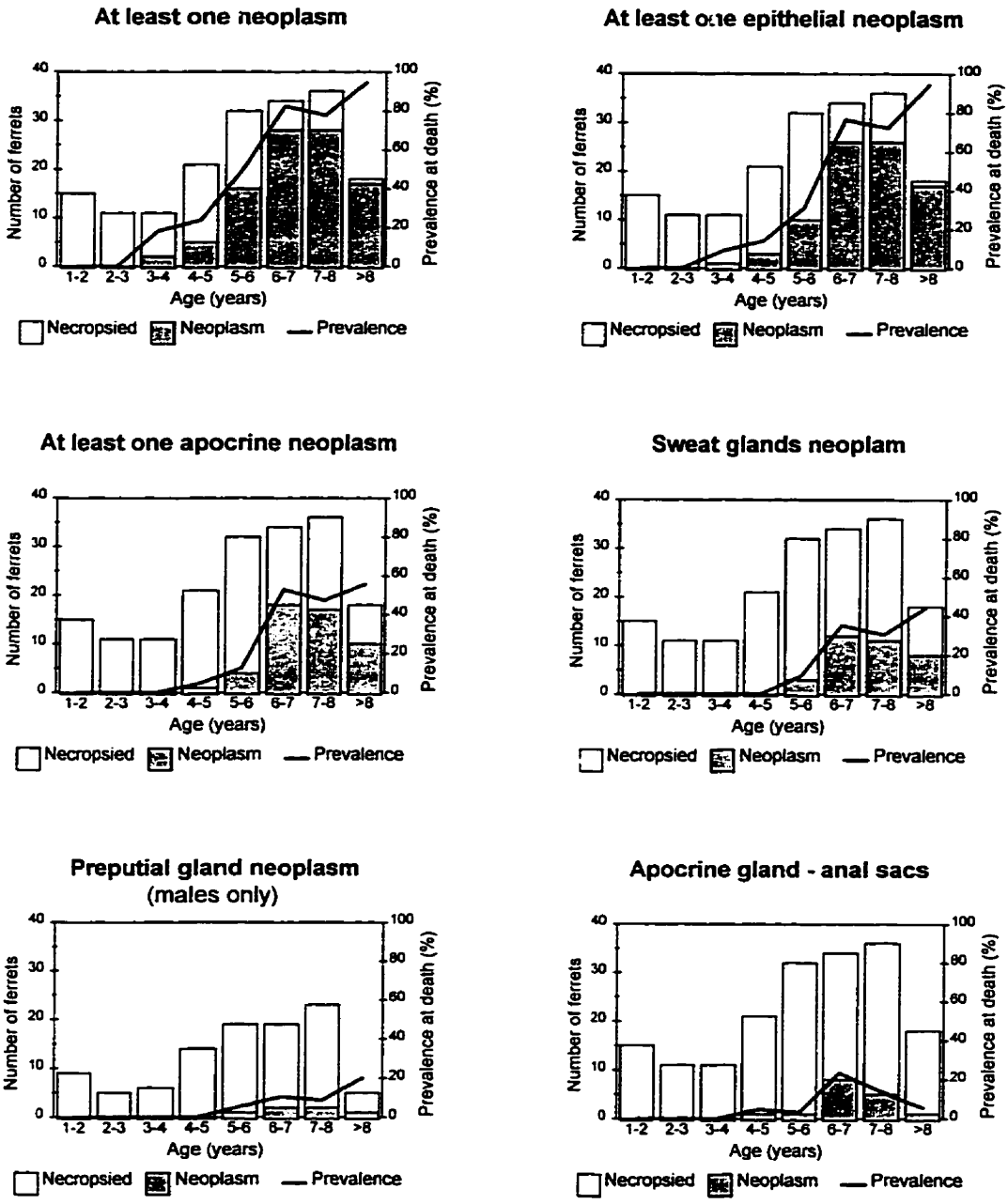
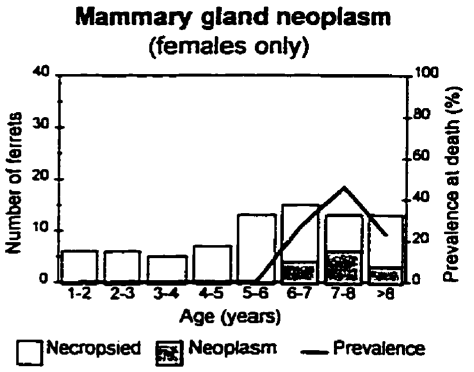
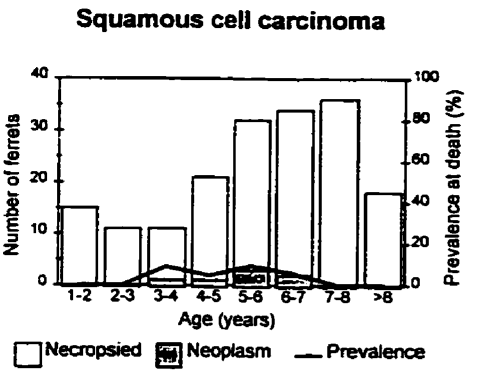
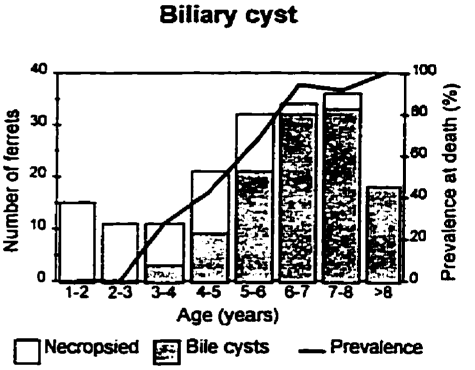
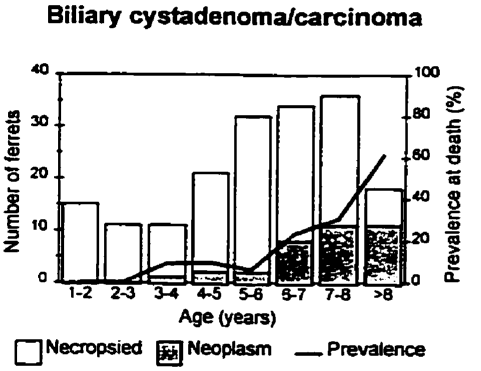


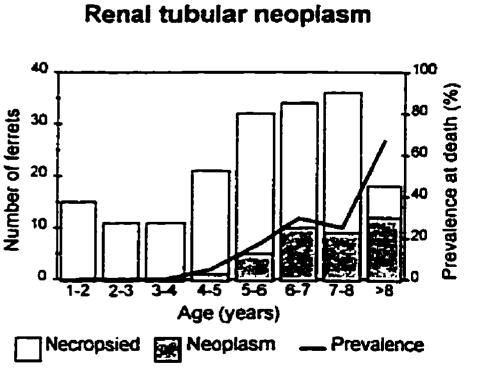
**Figure 4.14** (cont.)



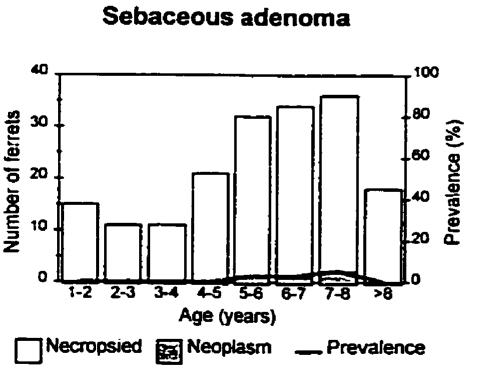



**Table 4.7** Number of neoplasms and invasive neoplasms per ferret.

| Neoplasms | All neoplasms | | Invasive neoplasms | |
|---|---|---|---|---|
| per animal | No. of cases | Rel. frequency (%) | No. of cases | Rel. frequency (%) |
| 1 | 51 | 50[a] | 50 | 53.19 |
| 2 | 32 | 31.37 | 33 | 35.11 |
| 3 | 11 | 10.78 | 9 | 9.57 |
| 4 | 5 | 4.9 | 0 | 0 |
| 5 | 2 | 1.96 | 2 | 2.13 |
| 6 | 1 | 0.98 | 0 | 0 |
| **More than 1** | **51** | **50** | **45** | **46.81** |

[a] Indicates that 50% of the ferrets with neoplasia had only one tumour.

**Figure 4.15** Correlation between age at death and number of neoplasms per animals.

Spearman's correlation coefficient (r) = 0.66, p-value = 0.001.

**Table 4.8** Relationship between the tumour status of the parents and the frequency of each event of interest in their offspring (n = 116).

| Event of interest | Status of parents | | | p-values [a] |
|---|---|---|---|---|
| | None affected (%) | One affected (%) | Both affected (%) | |
| Biliary cysts | 66.67 [b] | 76.67 [b] | 81.93 [b] | 0.55 |
| All neoplasms | 100 | 67.74 | 67.86 | 1 |
| Epithelial neoplasms | 66.67 | 52.5 | 62.86 | 0.59 |
| Apocrine epithelial neo. | 30 | 32.1 | 26.67 | 0.91 |
| Biliary neoplasms | 37.93 | 23.88 | 15 | 0.17 |
| Renal neoplasms | 35.9 | 27.08 | 20.69 | 0.37 |
| Sweat gland neoplasms | 18.18 | 24.59 | - | 0.40 |
| Apocrine - anal neo. | 10 | 0 | - | 1 |

[a] Chi-square test or Fisher's exact test.
[b] Percentage of offspring affected by the event of interest in each group.

**Table 4.9** Relationship between the tumour status of the parents and the age at onset for each event of interest in their offspring.

| Event of interest (n) | Status of parents affected | | | p-values [a] |
|---|---|---|---|---|
| | None affected | One affected | Both affected | |
| Biliary cysts (92) | 7.35 ± 0.43 [b] | 6.91 ± 1.23 [b] | 6.73 ± 1.23 [b] | 0.76 |
| All neoplasms (77) | - | 7.01 ● 1.30 | 6.88 ± 1.25 | 0.76 |
| Epithelial neoplasms (68) | 6.29 ± 0.75 | 7.19 ● 1.26 | 7.06 ± 1.15 | 0.39 |
| Apocrine epithelial neo. (36) | 6.53 ± 1.12 | 7.41 ± 0.81 | 7.41 ± 0.61 | 0.06 |
| Biliary neoplasms (30) | 6.61 ± 1.45 | 7.61 ± 0.95 | 7.29 ± 1.19 | 0.14 |
| Renal neoplasms (33) | 7.41 ± 1.27 | 7.09 ± 1.21 | 7.47 ● 0.97 | 0.73 |
| Sweat gland neoplasms (25) | 7.25 ● 0.73 | 7.49 ± 0.97 | - | 0.53 |

[a] One-way analysis of variance (ANOVA).
[b] Age at onset in years (mean ± STD) for the event of interest in offspring of each group.

**Table 4.10** Logistic regression models for each event of interest vs. presence of biliary cysts or various types of neoplasia. Only the variables that were included in each model are presented.

| Events of interest | Variables included in the model | Parameter Estimate[a] | Standard Error | Odds Ratio | P-values |
|---|---|---|---|---|---|
| Biliary cysts | Date of birth[b] | 0.52 | 0.17 | 1.68 | 0.002 * |
| | Age | 1.60 | 0.25 | 4.95 | 0.0001 * |
| All neoplasms | Date of birth[b] | - 0.23 | 0.17 | | 0.179 |
| | Age | 0.96 | 0.19 | 2.62 | 0.0001 * |
| | % of life at Toronto[c] | 0.05 | 0.03 | 1.65 | 0.036 * |
| Epithelial neoplasms | Date of birth[b] | - 0.06 | 0.17 | | 0.735 |
| | Age | 1.15 | 0.21 | 3.15 | 0.0001 * |
| | % of life at Toronto[c] | 0.05 | 0.02 | 1.65 | 0.038 * |
| Apocrine epith. neoplasms | Date of birth[b] | - 0.03 | 0.18 | | 0.73 |
| | Age | 0.97 | 0.22 | 2.64 | 0.0001 * |
| | % of life at Toronto[c] | 0.07 | 0.02 | 2.01 | 0.035 * |
| Biliary neoplasms | Date of birth[b] | 0.02 | 0.17 | | 0.918 |
| | Age | 0.72 | 0.2 | 2.05 | 0.0002 * |
| Renal neoplasms | Date of birth[b] | - 0.20 | 0.19 | | 0.295 |
| | Age | 0.75 | 0.21 | 2.12 | 0.0004 * |
| | % of life at Toronto[c] | 0.03 | 0.01 | 1.35 | 0.013 * |

**Table 4.10 cont.**

| Events of interest | Variables included in the model | Parameter Estimate [a] | Standard Error | Odds Ratio | p-values |
|---|---|---|---|---|---|
| Sweat gland neoplams | Sex | 1.49 | 0.56 | 4.44 | 0.007 * |
| | Date of birth [b] | 0.09 | 0.23 | | 0.69 |
| | Age | 1.20 | 0.29 | 3.32 | 0.0001 * |
| | % of life at "Toronto" [c] | 0.04 | 0.06 | 1.49 | 0.005 * |
| Apocrine - anal sac neoplasms | Sex | 2.80 | 1.14 | 16.44 | 0.014 * |
| | Age | 1.26 | 0.41 | 3.54 | 0.002 * |
| | Ratio first rel [d] | - 0.27 | 0.08 | 0.77 | 0.0014 * |
| | % of life at "Sybille" [c] | - 0.024 | 0.01 | 0.79 | 0.014 * |
| Mammary neoplasms [e] | Date of birth [b] | - 0.23 | 0.25 | | 0.377 |
| | Age | 0.57 | 0.29 | 1.77 | 0.046 * |

[a] Negative value for Parameter Estimate indicates a sparing effect.
[b] OR for "Date of birth" are for intervals of one year.
[c] OR for "% of life spent at..." are for intervals of 10%.
[d] Ratio first relative: Percentage of first generation relatives also affected by the event of interest. OR for "Ratio first rel" are for intervals of 1% interval.
[e] Analysis for mammary neoplasms in females of the study group.
* Statistically significant.

**Figure 4.16** Age distribution of fatal cases of neoplasia in male and female black-footed ferrets.

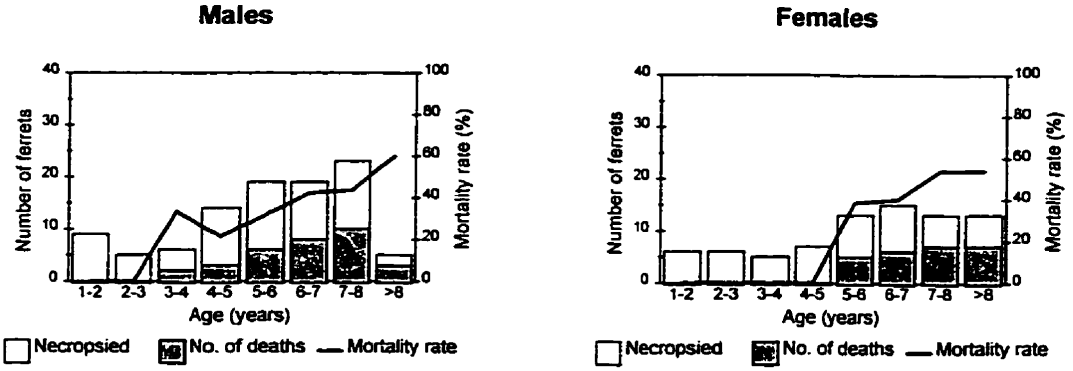



**Table 4.11** "Symptomatic ratios" and "fatality ratios" for biliary cysts and for the most common neoplasms.

| Events | No. of cases | Symptomatic[a] | | Fatal[b] | |
|---|---|---|---|---|---|
| | | No. | % | No. | % |
| Biliary cyst | 121 | 14 | 11.57 | 3 | 2.48 |
| Renal tubular neoplasm | 38 | 5 | 13.16 | 4 | 10.53 |
| Biliary cystadenoma/cystadenocarcinoma | 37 | 24 | 64.86 | 21 | 56.76 |
| Sweat gland neoplasm | 36 | 12 | 33.33 | 2 | 5.56 |
| Apocrine gland - anal sac neoplasm | 16 | 14 | 87.5 | 13 | 81.25 |
| Mammary gland neoplasm | 13 | 8 | 61.54 | 7 | 53.85 |
| Squamous cell carcinoma | 8 | 8 | 100 | 7 | 87.5 |
| Preputial gland neoplasm | 6 | 3 | 50 | 2 | 33.33 |
| Sebaceous adenoma | 5 | 4 | 80 | 0 | 0 |

[a] "Symptomatic ratios": Percentage of cases associated with clinical signs.

[b] "Fatality ratios": Percentage of animals affected by the condition that died or were euthanatized due to this condition.

**Table 4.12** Comparison of crude annual rates (CAR) of neoplasia for different species.

| Species | CAR of neoplasia per 100,000 | | | References |
|---|---|---|---|---|
| | All neoplasms | Invasive neoplasms | Fatal cases (mortality) | |
| Black-footed ferret[a] | 4458 | 3881 | 2360 | Present study |
| Domestic ferret[b] | 300 - 2000 | | | Beach et al. (1993) |
| Domestic ferret[c d] | 1724 | | | Priester et al. (1980) |
| Mink[e] | 0 | 0 | 0 | Schneider et al. (1993) |
| Human - male[f] | | 438 | 237 | Statistics Canada (1997b) |
| Human -female[f] | | 342 | 153 | Statistics Canada (1997b) |
| Human - both sex[g] | | 396 | 171 | Ries et al. (1997a) |
| Dog[c] | 3329 | | | Priester et al. (1980) |
| Cat[c] | 1448 | | | Priester et al. (1980) |
| Horse[c] | 1430 | | | Priester et al. (1980) |
| Ox[c] | 1365 | | | Priester et al. (1980) |

[a] CAR for the four last years of the study (1994-1997).
[b] Estimates of CAR calculated with data extracted from the manuscript.
[c] Crude annual rate of tumours per 100,000 in hospital-based populations.
[d] Based on only two cases of neoplasms.
[e] Adult females from one mink farm in Ontario.
[f] Estimates of crude annual rate of invasive neoplasms per 100,000 in the Canadian population for 1997.
[g] Crude annual rate of invasive neoplasms per 100,000 in a subsample of the American population for 1994.

**Table 4.13** Comparison of annual rates of neoplasia in different species standardized to life span in people (LSAAR).

| Species | LSAAR of neoplasia per 100,000 | | | References |
|---|---|---|---|---|
| | All neoplasms | Invasive neoplasms | Fatal cases (mortality) | |
| Black-footed ferret[a] | 446 | 388 | 236 | Present study |
| Domestic ferret[b] | 30 - 200 | | | Beach et al. (1993) |
| Domestic ferret[c] | 172 | | | Priester et al. (1980) |
| Mink[d] | 0 | 0 | 0 | Schneider et al. (1993) |
| Human - male[e] | | 438 | 237 | Statistics Canada (1997b) |
| Human -female[e] | | 342 | 153 | Statistics Canada (1997b) |
| Human - both sex[f] | | 396 | 171 | Ries et al. (1997a) |
| Dog[c] | 587 | | | Priester et al. (1980) |
| Cat[c] | 255 | | | Priester et al. (1980) |
| Horse[c] | 421 | | | Priester et al. (1980) |
| Ox[c] | 241 | | | Priester et al. (1980) |

[a] LSAAR for the four last years of the study (1994-1997).
[b] Estimates of LSAAR calculated with data extracted from the manuscript.
[c] LSCAR of cancer per 100,000 in hospital/clinic populations of dogs, cats, horses, and domestic ferrets.
[d] Adult females from one mink farm in Ontario.
[e] Estimates of crude annual rate of invasive neoplasms per 100,000 in the Canadian population for 1997.
[f] Crude annual rate of invasive neoplasms per 100,000 in a subsample of the American population for 1994.

**Table 4.14** Prevalence of neoplasia at death reported for different species, and probability of developing a neoplasm of people.

| Species | Prevalence / probability of neoplasia (%) | | | References |
|---|---|---|---|---|
| | All | Invasive | Fatal | |
| Black-footed ferret | 55.4 | 51.1 | 33.7 | Present study |
| Domestic ferret[a] | 12 | | | Li et al. (1998) |
| Mink[b] | | | 0 | Schneider et al. (1993) |
| Mink[c] | 0 | 0 | 0 | Beek et al. (1990) |
| Mink[d] | > 4.7 | | | Hadlow (1984) |
| Zoo mammals[e] | 2.75 | 1.25 | 1.5 | Effron et al. (1977) |
| Zoo mammals[e] | 8.99 | | | Lombard et al. (1959) |
| Zoo mammals[e] | 2.94 | | | Appleby (1969) |
| Zoo mammals[e] | 10.43 | | | Montali (1980) |
| Rat[f] | 46.5 | | 18.5 | Chandra et al. (1992) |
| Human - male[g] | | 40.9 | 26.9 | Statistics Canada (1997b) |
| Human - female[g] | | 35 | 22.4 | |

[a] Domestic ferrets presented at different teaching hospitals.
[b] Adult females from one mink farm in Ontario.
[c] Farm mink of various ages.
[d] Estimates for mink kept for studies on slow viral diseases. Represents only nonhematopoietic/nonlymphoreticular neoplasms.
[e] Various species of mammals from zoological collections
[f] Sprague-Dawley rats used as controls for toxicology studies.
[g] Probability of developing invasive neoplasms and of dying from neoplasia for the Canadian population for 1997.

**Table 4.15** Life-span adjusted annual rates, prevalences at death, and relative frequencies for renal tubular neoplasms.

| Species | Renal tubular neoplasms | | |
|---|---|---|---|
| | LSAAR[a] | Prevalence[b] (%) | Frequency[c] (%) |
| Black-footed ferret[d] | 163 | 20.65 | 20.54 |
| Domestic ferret[e] | | | 0.13 |
| Mouse[f] | | 40 | |
| Mouse[g] | | 0.01 to 7.58 | |
| Zoo mammals[h] | | 0.03 | 1.03 |
| Rat[i] | | 0.04 | 0.04 |
| Rat[j] | | 24 - 51 | |
| Dog[k] | 1.2 | 0.08 | |
| Cat[k] | 0.56 | 0.05 | |
| Horse[k] | 0 | 0.08 | |
| Ox[k] | 0 | | |
| Human[l] | 9.4 | | 2 |

[a] Crude annual rate per 100,000 adjusted to human life-span.
[b] Proportion of animals examined at death that had a renal tubular neoplasm.
[c] Frequency of renal tubular neoplasms as a proportion of all tumours encountered at death.
[d] LSAAR for the four last years of the study (1994-1997) for renal tubular neoplasms.
[e] Domestic ferrets presented to teaching hospitals (Li et al., 1998).
[f] One strain of BALB/cf/Cd mice (Claude, 1958).
[g] Summary for different strains of mice (Sass, 1986).
[h] Various species of mammals from two zoological collections (Effron et al., 1977).
[i] Sprague-dawley rats used as controls for toxicology studies (Chandra et al., 1992).
[j] One strain of Wistar rats (Eker, 1954).
[k] LSAAR for hospital/clinic populations, included adenoma and adenocarcinoma of the kidney (Priester and McKay, 1980). Prevalence at death (Haschek et al., 1981).
[l] LSAAR for a subsample of the American population for 1994, included all tumours in the kidney of epithelial origin (Anonymous, 1997a). Frequency of renal neoplasms (Kosary and McLaughlin, 1993).

**Table 4.16** Life-span adjusted annual rates, prevalences at death, and relative frequency of mammary gland neoplasms.

| Species | Mammary gland neoplasms | | |
|---|---|---|---|
| | LSAAR[a] | Prevalence[b] (%) | Frequency[c] (%) |
| Black-footed ferret[d] | 63 | 7.07 | 7.03 |
| Domestic ferret[e] | | | 0.31 |
| Zoo mammals[f] | | 0.19 | 6.52 |
| Rat[g] | | 16.34 | 18.81 |
| Dog[h] | 62.6 | | 10.00 - 44.00 |
| Cat[h] | 13.3 | | 10.94 |
| Horse[h] | 0.19 | | |
| Ox[h] | 0.16 | | |
| Human[i] | 60 | | |

[a] Crude annual rate per 100,000 adjusted to human life-span.
[b] Proportion of animals examined at death that has a neoplasm.
[c] Frequency of mammary neoplasms as a proportion of all tumours encountered at death.
[d] LSAAR for the four last years of the study (1994-1997), included mammary adenoma, adenocarcinoma, and carcinosarcoma.
[e] Domestic ferrets presented to teaching hospitals; all mammary tumours (Li et al., 1998).
[f] Various species of mammals from two zoological collections; all mammary tumours (Effron et al., 1977).
[g] Sprague-dawley rats used as controls for toxicology studies; included mammary fibroadenoma, adenoma and adenocarcinoma (Chandra et al., 1992).
[h] LSAAR for hospital/clinic populations (Priester and McKay, 1980). Includes mammary adenocarcinoma, adenoma, and mixed tumours; frequencies for dogs cited in Moulton (1990b), and frequencies for cats reported by Schmidt et al. (1967).
[i] LSAAR for a subsample of the American population for 1994 (Anonymous, 1997a).

# 5 DISCUSSION

## 5.1 Comparative epidemiology of neoplasia in black-footed ferrets

### 5.1.1 Comparison with other populations

Compared to other species, captive black-footed ferrets seem to have a very high crude annual incidence of neoplasia (Table 4.12, page 88), which supports their reputation as "tumour-factories". However, these figures should be interpreted with caution. It is not surprising to have a high annual incidence of an age-related phenomenon like neoplasia in a species with a relatively short life expectancy such as the ferret. The crude annual rate measures the occurrence over an interval of one year. This interval represents approximately an eighth of the life expectancy of a ferret, but only a very small fraction of a human life. Consequently, to compare annual rates of neoplasia between different species, the relative life span of these species should be taken into account. The annual rate standardized for the life span (LSAAR) therefore represents a better indicator of the relative susceptibility to neoplasia for each population (Table 4.13, page 89).

The incidence of tumours in black-footed ferrets is higher than the crude annual rate available for its close relative, the domestic ferret. However, figures given by Priester et al. (1980) are based on a population of ferrets composed of younger animals than our population (no ferrets older than 4-yrs-old). The same limitation also applies to the population of mink reported by Schneider et al. (1993).

When adjusted to the human life-span the incidence of neoplasia in black-footed

ferrets is in the same range as the Canadian and American human populations, and domestic animals. However, the incidences for domestic animals are based on hospital populations, and therefore may not reflect the true incidence in the overall population. Hence, when corrected for life-span, the occurrence of neoplasia in the captive population of black-footed ferrets is high, but not exceptionally so, in comparison with other species where populations are not exposed to predation, or culling, are well-nourished, and allowed to live all their life-span.

### 5.1.2 Prevalence of neoplasia at death in black-footed ferrets

More than half of the adult ferrets that were necropsied were affected by at least one neoplasm, and over 30% of the mortalities in adult ferrets were attributed to neoplastic disease.

The prevalence of neoplasia at death in black-footed ferrets is far higher than information published for domestic ferrets (Li et al., 1998), and for two populations of mink (Beek et al., 1990; Schneider and Hunter, 1993) (Table 4.14, page 90). However, the difference in age distribution between our population and these three populations of mustelids limits the comparison. Indeed, mink bred for their pelts rarely live more than four years (Padgett et al., 1968), and are therefore unlikely to develop neoplastic disease. In contrast, the age at death of the population of mink studied by Hadlow (1984) was probably comparable to ours, since they were used in studies of slow viral diseases, and therefore lived to advance ages. Unfortunately, the only prevalence of neoplasia at death available from this population excluded haematopoietic and lymphoreticular neoplasms.

Nevertheless, since neither haematopoietic nor lymphoreticular neoplasms have been observed in the black-footed ferrets, non-haematopoietic/non-lymphoreticular neoplasms were 10 times more common at death in the captive population of black-footed ferrets then in this population of mink.

Reported prevalences at death of neoplastic diseases in mammals held in zoological collections varied from 2.75% to 10.43% (Table 4.14, page 90). The prevalence observed in the population of black-footed ferrets is at least five times higher then these estimates. The prevalence of neoplasia at death in black-footed ferrets is in the same range as that described in Sprague-Dawley rats used as non-exposed controls in toxicologic studies. However, deaths attributed to neoplasia are more frequent in black-footed ferrets.

The data available for people represent the probability for an individual to develop an invasive neoplasm during his/her life, or die of neoplasia. These give a good estimate of the prevalence of neoplasia at death and of the prevalence of deaths attributed to neoplasia. Based on our findings, the chances of developing an invasive neoplasm or of dying of neoplasia are slightly higher in the black-footed ferret population, but in the same range as that described in people.

### 5.1.3 Neoplastic phenotypes in black-footed ferrets and other mustelids

Twenty-eight (28) different phenotypes of neoplasms were encountered in our population of black-footed ferrets. Epithelial tumours comprised more than 90% of all cases (Table 4.5, page 78), and most of these cluster in three syndromes: renal tubular

neoplasms, cystic biliary neoplasms, and tumours of the various apocrine glands. The range of neoplasms in this species is very different from that in its close relative the domestic ferret, where lymphomas, insulinomas, or adrenocortical tumours account for 50% to 80% of the tumours reported (Brown, 1997; Li et al., 1998), in contrast with the situation in black-footed ferrets, where none of these neoplasms were seen. Conversely, the eight most common neoplasms encountered in black-footed ferrets, comprising 85.9% of the tumours diagnosed in this population, have been described in domestic ferrets only occasionally.

The absence of adrenocortical tumours in black-footed ferrets is consistent with the hypothesized link between endocrine neoplasms and premature gonadectomy in domestic ferrets (Rosenthal et al., 1993). A proportion of the cases of lymphoma in domestic ferrets probably have a viral etiology (Brown, 1997). The absence of lymphomas in our population suggests either a lack of exposure to this or a related virus, or a lack of pathogenic potential for such viruses in this species.

## 5.2 Renal tubular neoplasms

### 5.2.1 Pathology

All the neoplasms encountered in the kidneys of black-footed ferrets were histologically similar; a distinction between adenomas and carcinomas could not be made, and consequently they are considered most likely part of the same process. These tumours usually were incidental postmortem findings, associated clinical disease being present in only three of the 38 cases. Haematuria, regularly described in people

(Bennington and Beckwith, 1975), was not reported in any of our animals.

Multiple renal tumours were common, and bilateral in at least 18.4% of the cases. This figure is much higher than in people, where bilateral renal tumours have a prevalence of 0.5 to 1.5% (Bennington and Beckwith, 1975). Multiple renal tubular neoplasms rarely have been described in domestic species, with the exception of cattle (Kelley et al., 1996). Metastases were detected in only one of 38 ferrets , and metastatic rates for this neoplasm also are low in mice (Sass, 1986) and rats (Bannasch and Zerban, 1986), though they are relatively high in dogs (Lucke and Kelly, 1976) and people (Ritchie and deKernion, 1987). A strong correlation between metastatic rate and size of the primary tumour has been reported in people (Bennington and Beckwith, 1975), and the single renal tumour with metastases in this study was one of the largest encountered. However, larger masses, without metastases, were seen in two other cases.

The generally low mitotic index is consistent with the slow growth and low metastatic rates of these tumours. Osseous metaplasia, evident in 83% of the ferret tumours, has also been described occasionally in human renal tumours (Bennington and Beckwith, 1975), but to our knowledge, not in such tumours in other species. Osseous metaplasia also was observed in the renal cortex of three ferrets without renal tumours, and was common in other neoplasms in this species.

### 5.2.2 Epidemiology

Renal tubular neoplasms occurred in 20.7% of the black-footed ferrets examined, and at an annual incidence of 1.6% during the four last years of the study. With the

exception of two strains of rodents, in which inheritance has been demonstrated (Eker, 1954; Claude, 1958), tumours of the renal cortex are relatively rare in other species, including people, domestic species and laboratory animals (Table 4.15, page 91). Renal tubular neoplasms are rare in domestic ferrets, where they have been reported on only four occasions (Symmers and Thomson, 1953; Li et al., 1998). This contrasts with the occurrence in the captive population of black-footed ferrets, and suggests that the "Meeteetse" population is predisposed to develop renal tubular neoplasms. This tumour was not observed in the five black-footed ferrets from the South Dakota population that died at an old age (Carpenter et al., 1981), but renal tumours have been diagnosed in Siberian polecats kept at the University of Wyoming (Williams et al. 1988).

Male black-footed ferrets were not affected by renal neoplasms more than females although a sex predisposition of males for renal carcinomas has been described in dogs (Lucke and Kelly, 1976), mice (Sass, 1986), and people (Anonymous, 1997a). A statistically significant association was seen between the development of renal tubular neoplasms and aging. This is obviously not uncommon for neoplastic diseases.

"Time spent at Toronto" was the only other variable associated with an increased risk of developing renal tubular neoplasms. This increase in risk with the time spent at the Toronto Zoo is most likely due to a higher sensitivity in the detection of these neoplasms at this institution, since the principal investigator of the present study (S.L.) has been at the Toronto Zoo for the last three years, and kidneys from black-footed ferrets dying at this institution were therefore thoroughly examined. Hence, the prevalence at death for renal tubular neoplasms reported in this study likely is a minimum figure, since

small renal tumours may have been missed in other institutions.

### 5.2.3 Risk factors and possible etiologies

The etiology of spontaneous renal tubular neoplasms is unknown (Nielsen and Moulton, 1990). Predisposing factors for renal tumours in people include cigarette smoking, obesity, and exposure to petroleum products [reviewed by Tavani et al. (1997)]. Black-footed ferrets, like many zoo animals, are frequently overweight. However, due to the lack of precise information regarding the body condition of these ferrets, an association between obesity and renal tubular neoplasms could not be explored explicitly. Numerous xenobiotic compounds have been shown experimentally to induce renal epithelial tumours in laboratory animals [for a review see Sass (1986)]. High rates of renal carcinomas have also been described in a natural setting in free-ranging rats exposed to lead (Kilham et al., 1962). The absence of an "institution-effect" on occurrence of renal tubular neoplasms in black-footed ferrets does not favour the implication of local environmental factors in the development of this condition. In addition, lead was not detected in the kidneys of any of the four animals assessed.

The uneven sex distribution in several species, the association with obesity observed in people (Tavani and La Vecchia, 1997), and the induction of renal carcinomas in hamsters with estradiol injections (Reznick and Schuller, 1979) suggest a possible hormonal association in the etiology of this tumour. Multiparity has been associated with some elevation in risk in women (Tavani and La Vecchia, 1997). The absence of sex predisposition and the lack of a relationship between renal tubular neoplasms and

reproductive parameters in our study do not support a potential hormonal influence in the development of this condition in black-footed ferrets.

A high incidence of renal neoplasia caused by a herpesvirus has been described in leopard frogs (Granoff, 1973), and occasional cases of renal adenomas have been associated with poxvirus infections in squirrels (O'Connor et al., 1980). Consequently, the possibility of a viral etiology for these tumours in black-footed ferrets can not be ignored. However, the absence of temporal or geographical case-clustering, and the absence of cases in young and middle-aged animals, would be unusual for a virus-induced neoplasm, and inclusion bodies, suggestive of certain viral etiologies, were not recognized.

Hereditary renal tubular neoplasms have been proposed in a group of rhesus macaque (*Macaca mulata*) at the Philadelphia Zoo (Ratcliffe, 1940), and have been reported in German shepherd dogs (Moe and Lium, 1997), in two laboratory strains of rodents (Eker, 1954; Claude, 1958), and in people with Hippel-Lindau disease (Fleming, 1997). The absence of patterns of Mendelian inheritance for renal neoplasia in black-footed ferrets (Table 4.9, page 84) does not support a hereditary syndrome due a to single-gene disorder in this species. In addition, the advanced age at onset is unusual for a hereditary tumour.

Nevertheless, several elements support a possible familial predisposition of our population of ferrets to this, most likely, multifactorial syndrome. These include: the exceptionally high occurrence of this tumour without any obvious predisposing factors; the common multifocal and bilateral manifestation of this neoplasm; the limited genetic

heterogeneity of this population (Russell et al., 1994); the extreme rarity of this neoplasm in domestic ferrets; and the absence of similar cases in black-footed ferrets from South Dakota (Carpenter et al., 1981). However, anecdotal reports of histologically similar renal neoplasms in Siberian polecats suggest that this neoplastic syndrome is not restricted to this group of black-footed ferrets.

Our study failed to show any effect of inbreeding on the risk of developing renal tubular neoplasms, and evidence of familial clustering could not be demonstrated. However, the high level of genetic homogeneity in this population might explain these outcomes.

### 5.2.4 Recommended clinical approach

Renal masses, detected either by radiography or palpation, should strongly suggest a renal tubular neoplasm in adult black-footed ferrets. Nephrectomy is the recommended treatment for this tumour in people (Bennington and Beckwith, 1975), and dogs (Lucke and Kelly, 1976). However, due to the common bilateral distribution, the slow growth habit, and the very low metastatic rate, the potential benefit of this invasive surgery in black-footed ferrets is questionable. We believe that in most cases, surgery should not be attempted, specially in animals that are close to the maximum life expectancy. Ferrets with renal tumours have been asymptomatic for several months following the diagnosis, and most of them will die of another cause.

Since the etiology of this tumour remains unclear, methods of prevention are unknown. However, weight control could potentially have a beneficial effect, since

overweight is a predisposing factor for this neoplasm in people (Tavani and La Vecchia, 1997), and rats (Turnbull et al., 1985).

## 5.3 Biliary dysplastic and neoplastic conditions

### 5.3.1 Pathology

Three cystic conditions of the liver, intrahepatic biliary cyst, biliary cystadenoma, and biliary cystadenocarcinoma, were encountered in a large proportion of the ferrets examined.

Intrahepatic biliary cysts were very common, but were seen only in ferrets over three years of age. The size and the number of these cysts increased with age. Intrahepatic biliary cysts could remain occult for several years, but occasionally were associated with clinical signs following rupture or secondary bacterial infection.

Neoplasms of the intrahepatic bile ducts also were very common in our population. The presence of both benign and malignant cellular phenotypes in most cases of biliary cystadenocarcinoma, and their overall histological similarity to cystadenomas, strongly suggest that they are an extension of the same process. The most striking feature of these tumours was their close association with biliary cysts; neoplastic growths clearly originating from the epithelial lining of these cysts, and they were seen in 31% of the ferrets with biliary cysts. Similar associations between intrahepatic biliary cysts and cystadenomas/carcinomas have been reported in people (Ishak et al., 1977) and cats (Adler and Wilson, 1995), and have been produced experimentally in rats exposed to aflatoxin (Cruickshank and Sparshott, 1971), or to N-nitrosodienthylamine and

phenobarbital (Duran et al., 1992). The presence of cholangiohamartoma-like lesions (Redston and Wanless, 1996), and of clusters of proliferating immature cholangiolar cells suggests a possible disruption in the homeostasis of the cholangiocellular elements leading to the development of biliary cysts and derived tumours.

Biliary cystadenocarcinomas were fast growing, markedly invasive malignancies. Most cases were associated with clinical signs, abdominal distention by ascitic fluid being the most common antemortem finding. Metastases were detected in approximately half of the cases, peritoneal serosa being the most common site of implantation. Metastatic neoplasms usually had higher mitotic indices and a higher degree of anisokaryosis than non-metastatic tumours. However, this relationship was not consistent. As with the renal neoplasms, osseous metaplasia was common in these tumours.

### 5.3.2 Epidemiology

Neither biliary cysts nor cholangiocellular neoplasms were reported in the five aged black-footed ferrets from the South Dakota population (Carpenter et al., 1981). Since they were believed to be at least 6-yr-old, the absence of these cystic anomalies in this population contrasts with the situation in the Meeteetse population, where prevalences at death for biliary cysts and biliary cystic neoplasms in ferrets of similar age (≥6-yr-old) were 94% and 34% respectively. Similar cystic neoplasms of the liver were diagnosed in Siberian polecats kept for research at the University of Wyoming (Williams et al. 1988). To our knowledge, neither biliary cysts nor biliary cystadenomas/carcinomas have been described in domestic ferrets, and neoplasms of the biliary tree have been

reported on only three occasions (Symmers and Thomson, 1953; Li et al., 1998).

Biliary cystadenomas and cystadenocarcinomas are rare in people (Cruickshank and Sparshott, 1971) and in domestic animals (Popp, 1990). The situation observed in the Meeteetse population, where 20% of the animals necropsied were affected by biliary cystic neoplasms, is consequently remarkable.

The prevalences at death of biliary cysts and cystic biliary neoplasms in our study progressively increased with age, reaching 100% and 60% respectively in animals over 8-yr-old. Statistically significant associations were observed between age and biliary cysts, and age and cystic biliary neoplasms. Similar age relationships have been described in cats (Adler and Wilson, 1995) and people (Ishak et al., 1977) with cystic cholangiocellular neoplasms. The likelihood of having biliary cysts also increased with the date of birth. The significance of this finding is unknown. Variables other than age had no detectable effect on the presence of biliary neoplasms (Table 4.10, page 85).

### 5.3.3 Risk factors and possible etiologies

Biliary tumours in black-footed ferrets appear to originate in intrahepatic biliary cysts, and their high prevalence at the time of death is likely a consequence of the very high prevalence of biliary cysts. The etiology of these hepatic cysts remains unclear, but might be related to an anomaly in the regulation of cholangiocellular differentiation, proliferation and maturation. This defect could be either a congenital syndrome or be the result of the exposure to mutagenic agents.

Congenital childhood and adult polycystic diseases in people, which are,

respectively, autosomal recessive and autosomal dominant hereditary disorders, are characterized by the development of intrahepatic and renal cysts [for a review see Klatskin et al. (1993)]. In the adult form, renal cysts are seen in 50% of the cases with hepatic cysts. In our study, isolated renal cysts were detected in only two ferrets with biliary cysts. The late clinical presentation of biliary cysts in our animals would be unusual for a congenital problem, but might be due to a very slow progression of the lesions, and in adult polycystic disease in people, clinical manifestations are delayed to the fourth and fifth decades. The cysts in adult polycystic disease of people, like the biliary cysts in black-footed ferrets, are lined by cuboidal or flattened epithelium and do not contain bile. Congenital hepatic polycystic anomalies have also been described in cats, piglets, and dogs (Kelly, 1991).

Cholangiohamartomas are frequently seen in both childhood and adult polycystic disease, and it has been proposed that biliary cysts are formed by the progressive dilation of these aberrant complexes of bile ducts (Drèze et al., 1995; Redston and Wanless, 1996). Interestingly, similar hamartomatous lesions were observed in the liver of 21% of the black-footed ferrets with biliary cysts. Since only limited tissue was available for microscopic examination in several cases, this represents a minimum figure. The absence of cholangiohamartoma-like lesions in the liver of ferrets younger than five years of age does not favour the congenital hypothesis, and would suggest that these biliary lesions are acquired. However, due to the limited number of hepatic tissue sections examined from young animals, this observation should be interpreted with caution.

The very high occurrence of biliary cysts in black-footed ferrets (100% in animals

older than 8-yr) would be quite unusual for a hereditary disease, and the absence of obvious patterns of Mendelian inheritance suggests that this syndrome is not due to a single-gene disorder.

Biliary cysts could also be acquired. Intrahepatic biliary cysts are occasionally seen in older rats, in which they are believed to be a degenerative change associated with aging (Eustis et al., 1990). Biliary cysts and biliary neoplasms have been induced experimentally in rats exposed to aflatoxin (Cruickshank and Sparshott, 1971), but not in other species (Kelly, 1991). Consequently, the possibility that the cystic disease observed in our study is acquired cannot be ruled out, and the age distribution of this syndrome would be consistent with a cumulative effect of exposure to endogenous or exogenous compounds capable of initiating proliferation of the biliary epithelium. Repetitive insults by one or many such compounds could induce proliferation of the biliary elements which might lead to the formation of dysplastic cholangiolar structures, subsequent intrahepatic cysts, and derived tumours. However, the very high occurrence and the uniform distribution of this syndrome would imply significant exposure to a disrupting compound in all seven principal breeding facilities.

The theory that would best explain the very high occurrence and broad distribution of biliary cystic diseases in this population is based on a multifactorial model involving both genetic and environmental factors. In that circumstance, this population of ferrets might have a genetic defect associated with a low disease-threshold, or an unusual sensitivity of the biliary elements to environmental "cystogenic" compounds.

### 5.3.4 Recommended clinical approach

Detection of hepatic masses, either by abdominal palpation or radiography, strongly suggests the presence of biliary cysts or tumours. Additional diagnostic procedures (fine needle aspiration, laparotomy/laparoscopy) would be needed to discriminate neoplastic lesions from biliary cysts. Surgical resection of biliary cysts in people has been advocated to prevent secondary malignancy (Ishak et al., 1977). However, due to the very high occurrence of biliary cysts in black-footed ferrets, and their tendency to affect several hepatic lobes, such an approach is not feasible in this species. Although animals with biliary cysts may remain asymptomatic for years, neoplasia is a common complication of these biliary cysts. Excision of biliary tumours by surgical lobectomy could be attempted, but cystadenocarcinomas are usually too advanced at the time of the diagnosis to hope for a curative surgery. Since, in the vast majority of the cases, surgical treatment will not significantly improve the life span and the welfare of the affected animal, symptomatic animals with a diagnosis of biliary cystadenocarcinoma should be euthanatized.

## 5.4 Neoplasms of the apocrine and mammary glands

Neoplasms of the apocrine glands were common, and comprised four distinct syndromes: sweat gland neoplasms, preputial gland neoplasms, mammary gland neoplasms, and adenocarcinomas of the apocrine gland of the anal sacs.

### 5.4.1 Neoplasms of the sweat gland

#### 5.4.1.1 *Pathology*

Sweat gland tumours were mainly localized on the tail, where the dermis contains a very high density of apocrine glands. Based on their histologic features, adenomas and adenocarcinomas likely form a continuum. Neoplasms of the sweat gland usually were slow growing, and rarely were associated with clinical signs. Despite the relatively high degree of cellular anaplasia commonly observed in adenocarcinomas, metastases were only detected in two of 23 cases, in both of which high mitotic indexes were observed. The clinical presentation, the morphologic features, and the behaviour of these tumours in black-footed ferrets resembled descriptions for dogs and cats (Pulley and Stannard, 1990).

#### 5.4.1.2 *Epidemiology*

The occurrence of sweat gland tumours in our population of ferrets is very high. Sweat gland neoplasms were also described in two of the black-footed ferrets from the South Dakota population (Carpenter et al., 1981). In contrast, these tumours have been reported only occasionally in domestic ferrets (Miller et al., 1985; Brown, 1997; Li et al., 1998), and they are uncommon in dogs and cats and rare in other domestic animals (Pulley and Stannard, 1990).

Three variables were significantly associated with the development of sweat gland tumours in our study: sex, age, and "time spent at Toronto" (Table 4.10, page 85). Males were four times more likely to be affected than females, and a positive association with age was observed. Sweat gland neoplasia is actually a disease of old age, all ferrets

affected being older than 5-yr-old. As for the renal tubular neoplasms, the increase in risk associated with time spent at Toronto Zoo is most likely spurious, and due to a higher sensitivity in the detection of these tumours at this institution. Small tumours could easily be missed if a detailed examination of the tail were not done. As for the renal tumours, this suggests that the actual prevalence at death for sweat gland neoplasms is a minimum figure.

#### 5.4.1.3 *Risk factors and possible etiologies*

The etiology of these tumours, and the cause for the high occurrence in black-footed ferrets, are unknown. The presence of sweat gland adenocarcinomas in two of the five ferrets from South Dakota necropsied suggests that this neoplasm was also common in that distinct population, and supports a species predilection rather than a predisposition due to local inbreeding. In addition, the absence of pattern of Mendelian inheritance does not favour a genetic etiology. The predilection for males suggests a potential hormonal influence, and the rarity of these tumours in domestic ferrets, which are usually castrated at a young age, would be consistent with that hypothesis.

### 5.4.2 Neoplasms of the preputial gland

#### 5.4.2.1 *Pathology*

Preputial glands are composed of sebaceous and apocrine glands surrounding the urinary meatus. The size of these glands varies through the year, being larger during the breeding season. Due to their intimate anatomic association with the urinary meatus, and

their generally highly invasive behaviour, tumours affecting the apocrine component of the preputial gland were more frequently associated with clinical signs than sweat gland neoplasms. As for the sweat gland neoplasms, mitotic index seemed to be a relatively good indicator of invasive and metastatic potential of these neoplasms.

#### 5.4.2.2 *Epidemiology*

The prevalence at death for preputial gland tumours is a minimum figure, since, as for sweat gland neoplasms, small adenomas may have been missed during postmortem examinations. This tumour has not been reported in other species of mustelids, and has been described only rarely in domestic animals. Neoplasms of the preputial gland are common in rats (Reznik and Ward, 1981), where they originate in the sebaceous glands, in contrast to the apocrine origin of tumours in black-footed ferrets.

This tumour was too infrequent to permit any epidemiologic analysis, but it was mainly encountered in older ferrets.

#### 5.4.2.3 *Risk factors and possible etiologies*

The etiology of this neoplasm is unknown. However, the seasonal changes observed in the size of this gland suggest that the development of these neoplasms is under gonadotropic influences. The absence of this neoplasm in domestic ferrets (which are usually neutered) is consistent with this hypothesis.

### 5.4.3 Neoplasms of the mammary gland

#### 5.4.3.1 *Pathology*

Histologic features suggesting malignancy were observed in nine of the 13 neoplasms of the mammary gland reviewed. In spite of the relatively high degree of cellular anaplasia commonly seen in adenocarcinomas, these tumours were usually relatively well-circumscribed, and metastases were only detected in one adenocarcinoma. Mitotic index was not a good indicator of metastatic potential, and tubular neoplasms were usually more invasive than the papillary tumours. The two carcinosarcomas showed a very high degree of anaplasia and were markedly invasive.

#### 5.4.3.2 *Epidemiology*

Mammary gland tumours were relatively common in females, with a prevalence at death of 16%. One of the two aged females examined from the South Dakota population was also affected by a papillary adenocarcinoma of the mammary gland (Carpenter et al., 1981). Tumours of the mammary gland have been reported only occasionally in domestic ferrets. The incidence of mammary neoplasms in black-footed ferrets is similar to that in dogs and people, but the prevalence at death is lower than for laboratory rats (Table 4.16, page 92). Neoplasms of the mammary gland were only observed in females, and prevalences at death increased significantly with age (Table 4.10, page 85).

#### 5.4.3.3 *Risk factors and possible etiologies*

Risk factors for the development of mammary tumours could not be identified in

this population of black-footed ferrets. In women, obesity, long reproductive life, high parity, and young age at first child have all been linked to increased risks for mammary neoplasms (Willet, 1993). This relationship between reproductive history and mammary gland neoplasia strongly suggests that exposure to endogenous oestrogens plays a role in the formation of these neoplasms. In dogs, a similar hormonal etiology has been proposed (Schneider et al., 1969; MacEwen et al., 1982). Hormonal influences have also been suggested for domestic cats (Hayes et al., 1981), and in captive exotic felids (Harrenstien et al., 1996). The rarity of mammary tumours in domestic ferrets, which are usually spayed before the first oestrus, suggests a relationship between hormonal status and the risk of mammary neoplasms in mustelids, but mammary tumours were uncommon in intact mink (Hadlow, 1985). An association between the development of mammary neoplasms and reproductive variables could not be demonstrated in our population of black-footed ferrets. However, this may have been related to the limited number of cases available for analysis. Hence, the role of female hormones in the etiopathogenesis of mammary neoplasia in black-footed ferrets remains unclear.

Viral etiologies have been demonstrated for mammary tumours in mice (Gross, 1984), and have been speculated in cats (Hayes et al., 1981). An infectious etiology for the mammary tumours in black-footed ferrets cannot be ruled out, but is unlikely, considering the sporadic pattern observed in this population.

Genetic predispositions for tumours of the mammary gland are well recognized in women (Willet, 1993), and have been proposed for dogs (Schafer et al., 1998), and cats (Hayes et al., 1981). Dam #22, and seven of the 35 female black-footed ferrets descended

from her, developed mammary tumours, so that eight of the 13 cases of mammary tumours were observed in animals with a direct familial link. However, the model used to evaluate potential familial clustering failed to demonstrate such a pattern for this disease.

### 5.4.4 Neoplasms of the apocrine gland of the anal sacs

#### 5.4.4.1 *Pathology of neoplasms*

Adenocarcinomas of the apocrine gland of the anal sacs were usually very invasive tumours associated with high metastatic rates and a poor prognosis. The degree of anisokaryosis and the mitotic index were usually higher in metastatic tumours, but this association was not consistent, and cellular morphology could not be used as a prognostic indicator. As in several other neoplasms in this species, osseous metaplasia was occasionally observed in these tumours. Adenocarcinomas of the apocrine gland of the anal sacs have been reported in dogs (Meuten et al., 1981; Ross et al., 1991) and in a population of mink (Hadlow, 1985), and they too display aggressive behaviour. While these tumours are essentially glandular in black-footed ferrets and dogs, sarcomatous neoplastic elements forming a mixed pattern of tumour are commonly observed in mink.

Hypercalcemia and hypophosphatemia frequently have been described in dogs affected by this tumour (Meuten et al., 1981), but not in mink (Hadlow, 1985). Levels of calcium and phosphorous were within the reference range for the species in the three ferrets affected by an adenocarcinoma of the apocrine gland of the anal sacs for which data were available.

5.4.4.2 *Epidemiology*

An adenocarcinoma of the apocrine gland of the anal sacs also was diagnosed in one male from the South Dakota population, a different lineage (Carpenter et al., 1981), implying a species, rather than population, susceptibility to this tumour.

The prevalence at death observed in black-footed ferrets for this neoplasm (8.7%) is similar to the prevalence reported in a population of mink (7.5%) (Hadlow, 1985). As for mink and dogs, adenocarcinomas of the apocrine gland of the anal sacs were seen mainly in aged ferrets, and a statistically significant association between age and this tumour was evident. Sex also has an effect on the likelihood to develop this neoplasm, all but two cases being encountered in males, which contrasts with the strong female predisposition reported in dogs (Meuten et al., 1981; Ross et al., 1991) and mink (Hadlow, 1985). The reason for this discrepancy is unknown. Significant negative effects (a decrease in the risk of developing the tumour) were also observed for the variables "time spent at Sybille", and "ratio of full siblings with neoplasms of the apocrine gland of the anal sacs" (Table 4.10, page 85). The biological significance of these sparing factors is unclear.

5.4.4.3 *Risk factors and possible etiologies*

In our study, age at death and sex were the only factors than influence the probability of having this tumour. The predisposition for males suggests that the development of this tumour is hormonally driven.

### 5.4.5 Recommended clinical approach for neoplasms of the apocrine glands

Neoplasms of the apocrine glands were by far the most common cutaneous tumours observed in black-footed ferrets. In contrast to sebaceous adenomas, which were always ulcerated, only large apocrine tumours ulcerated. The recommended clinical approach for these neoplasms depends of their location.

Neoplasms of the sweat gland located on the tail are non-invasive, grow slowly, and despite a microscopic appearance frequently suggesting malignancy, have a very low metastatic rate (4.2%). Consequently, surgical excision of these tumours of the tail is advocated only if they are associated with local discomfort. In contrast, neoplasms of the sweat gland affecting other parts of the body, and neoplasms of the mammary gland should be considered as potentially malignant. "Non tail masses" should therefore be surgically removed as soon as possible and submitted for histopathology.

Due to their high invasive potential, and their location at the urinary meatus, neoplasms of the preputial gland should also be removed. Hypertrophy of the preputial gland, frequently observed during the breeding season, should not be mistaken for a neoplastic growth.

Peri-anal masses and infection of the anal sacs are highly suggestive of adenocarcinoma of the apocrine gland of the anal sacs. Since these tumours are markedly invasive and have a high metastatic rate, they should be surgically removed as soon as possible. However, they usually are already too large at the time of diagnosis to be completely excised. Metastases to regional lymph nodes have been seen with very small adenocarcinomas. Consequently, the peri-anal area should be carefully examined when

regional lymphadenopathy or distant metastases are detected.

The male predilection for the neoplasms of the sweat gland and of the apocrine gland of the anal sacs suggests a potential link with testicular hormonal stimulation. Since tumours of the preputial gland, apocrine gland of the anal sacs, and sweat gland of the tail are possibly hormonally driven, castration of post reproductive males might reduce the risk of development of these tumours.

Ovariectomy decreases the risk of mammary neoplasia in bitches, but only if performed early in reproductive life (Schneider et al., 1969). Consequently, even if mammary tumours in black-footed ferrets are influenced by the hormonal climate, ovariectomy of post-reproductive females is unlikely to influence their occurrence.

In summary, with the exception of non-ulcerated tumours affecting the tail, all cutaneous growths should be considered as potentially malignant and should therefore be investigated. The success of surgical therapy will rely on the early detection of these cutaneous masses. Thorough physical exams, with special attention to the peri-anal, mammary and preputial areas, should therefore by performed regularly in adult ferrets.

## 5.5 Oral squamous cell carcinoma

### 5.5.1 Pathology

Most of the squamous cell carcinomas occurred in the oral cavity. At this site, these fast-growing tumours were associated with massive local destruction of connective tissue and bone, but rarely metastasized. All the black-footed ferrets affected by this neoplasm either died or were euthanatized due to this malignancy. The gross

morphology, the histologic features and the behaviour of the oral squamous cell carcinomas in black-footed ferrets resemble descriptions in dogs and cats (Head, 1990).

### 5.5.2 Epidemiology

The LSAAR for oral squamous cell carcinomas in black-footed ferrets (32) was much higher than the LSAAR previously described in domestic animals (0.49 - 6.4) (Priester and McKay, 1980). This tumour has been only rarely reported in domestic ferrets. However, due to the limited number of cases seen in this study, this apparent species predilection should be interpreted with caution. The number of cases is also too small to allow any epidemiologic analysis. Ferrets affected by this condition were generally relatively young compared with others with tumours.

### 5.5.3 Risk factors and possible etiologies

It is tempting to link the development of these oral neoplasms with the high occurrence of periodontal disease observed in this species. However, oral squamous cell carcinoma is relatively uncommon in comparison with periodontal disease, which is seen in most aged black-footed ferrets. The relationship between squamous cell carcinomas and gingival disease also is unclear in dogs (Head, 1990).

### 5.5.4 Recommended clinical approach

Focal gingival hyperplasia is commonly seen in black-footed ferrets, usually associated with periodontal disease. However, squamous cell carcinomas should always

be considered in cases of gingival swelling, and affected mucosa should therefore be biopsied. Mandibular or maxillary osteolysis, detected by radiography, is highly suggestive of squamous cell carcinoma in this species, and due to the invasive nature of this cancer, the prognosis always should be guarded. Hemimandibulectomy could probably be attempted for localised unilateral mandibular tumours, but it has not been done in black-footed ferrets, and should be limited to valuable breeders. Bleomycin chemotherapy has been attempted on a case of oral squamous cell carcinoma in a domestic ferret with partial and only temporary remission (Hamilton and Morrison, 1991).

## 5.6 Sebaceous adenoma

### 5.6.1 Pathology

Neoplasms of the sebaceous glands encountered in black-footed ferrets from this population were all benign, although epidermal ulceration occurred in all cases. Histologic features and behaviour of these tumours were similar to descriptions in domestic species (Pulley and Stannard, 1990).

### 5.6.2 Epidemiology

Sebaceous adenomas were also encountered in black-footed ferrets from the South Dakota population (Carpenter et al., 1981), and are one of the most common tumours in domestic ferrets (Table 2.2, page 9). This neoplasm is also relatively common in dogs, with a predilection for females (Strafuss, 1976), that was not observed in our study. The

small number of cases available limited the epidemiological analysis.

### 5.6.3 Risk factors and possible etiologies

Risk factors and etiology for this tumour in black-footed ferret are unknown.

## 5.7 Other neoplasms

The high diversity of neoplasms detected in this small group of ferrets is noteworthy, as is the skewedness toward epithelial tumours. Beyond the neoplastic conditions described above, sporadic cases of 15 other types of neoplasms were diagnosed in this population (Table 4.5, page 78). Due to the small number of each type of neoplasm it is impossible to speculate on behaviour, epidemiology, risk factors, and possible etiology. However, some observations are of interest.

The two basal cell tumours diagnosed were found on the same animal. The rarity of this cutaneous neoplasm in this population of aged ferrets contrasts with its relative abundance in domestic ferrets (Parker and Picut, 1993). A basal cell tumour was also detected in one of the five "South Dakota" ferrets (Carpenter et al., 1981). The diagnosis of two cases of olfactory neuroblastoma, an extremely rare malignancy in animals (Zaki and Liu, 1974), in less than 200 postmortem examinations is very surprising. Most of the other sporadic tumours, such as haemangioma, haemangiosarcoma, fibroma, uterine leiomyoma, transitional cell carcinoma, seminoma, and interstitial cell tumour, have been occasionally reported in domestic ferrets. Consequently, the presence of occasional cases of these tumours in our population of mustelids is not exceptional.

## 5.8 The genetic hypothesis and multifactorial model

One of the primary objectives of the present study was to explore the potential link between the high incidence of neoplastic diseases and the low genetic diversity of this population.

The genetic heritage of the host could influence the likelihood of developing a neoplasm. As discussed in the literature review, familial clustering of neoplasia could be directly associated with single-gene disorders. The very high occurrence of neoplastic disease encountered, the advanced age at onset, and the absence of patterns of Mendelian inheritance do not support the hypothesis of a single-gene disorder for any of the neoplasms observed in this population of black-footed ferrets.

A multifactorial model combining environmental and genetic factors more plausibly explains the situation encountered in this population. In this model, the liability of the host to develop a neoplasm would depend on its response to carcinogens. Accordingly, the unusually high frequency of hepatic, renal and apocrine epithelial neoplasms in this population might be due to the fact that these animals share similar environments (diet, housing, husbandry), and similar genetic heritage. It is reasonable to believe that a genetically homogeneous population would have a stereotypic response when exposed to similar environmental carcinogens. These stereotypic responses would generate homogeneous "outcomes", or, in this population, high occurrence of neoplastic syndromes.

Different mechanisms could be involved in this possible genetically driven susceptibility to neoplasia. Inherited chromosomal alterations have been associated with

functional modifications of different enzymes involved in the activation of procarcinogens into carcinogens. These alterations would therefore increase the potency of specific carcinogenic compounds, and consequently be associated with an unusual susceptibility to certain neoplasms (Cotran et al., 1994). Another possible mechanism would involve the occurrence of genetic anomalies associated with the activation of proto-oncogenes or with functional alterations to cancer suppressor genes, which would promote the growth and survival of damaged cells with neoplastic behaviours (Cotran et al., 1994). Finally, genetic alterations in the repair mechanisms of DNA defects have been reported in certain inherited diseases such as xeroderma pigmentosum. In this syndrome, the presence of a inherited inability to repair DNA damage related to exposure to ultraviolet light is associated with a high incidence of cutaneous malignancy (Kraehn et al., 1995).

This multifactorial model is not supported because of the failure to detect familial clustering or association between neoplastic diseases and inbreeding. However, the very high level of genetic homogeneity in the study population, and the very high occurrence of the principal neoplastic syndromes might account for this failure.

## 5.9 Impact of neoplastic diseases on the captive propagation of black-footed ferrets and on wild populations

Neoplasia accounted for more than 30% of the deaths in adult ferrets. The production of offspring decreases dramatically with age, from 1.54 weaned kits per female under three years of age, to 0.26 weaned kits per female three years of age and older (Thorne and Russell, 1995). Since mortalities due to neoplasms were exclusively encountered in females older than five years of age (Fig. 4.16, page 87), and therefore in their post-reproductive life, the impact of these conditions on the captive propagation of this species is probably limited. Analysis of the studbook revealed that males may remain fertile up to the sixth year of their life, while fatal cases of neoplasia have been recorded in males from three years of age (Fig. 4.16, page 87). Neoplastic disease consequently is associated with the premature loss of some reproductive males. Because of the polygamous nature of male ferrets, these losses probably are not associated with a decline in production of young, but might contribute to a decrease in genetic diversity.

The life span for free ranging black-footed ferrets is undoubtedly shorter than for captive animals; animals older than four years of age have been observed very rarely in the wild (Miller et al., 1996). Consequently, neoplastic diseases would be a rare event in the wild population, and therefore would be an insignificant cause of mortality.

The post-reproductive age class affected by tumours mitigates against genetic selection for resistance to neoplasia.

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**APPENDIX I** - Institutions involved in the black-footed ferret breeding program.

National Black-Footed Ferret
Conservation Center
410 E. Grand Avenue, Suite 315,
Laramie, Wyoming, 82070

Cheyenne Mountain Zoological Park
4250 Cheyenne Mount. Zoo Road
Colorado Springs, CO, 80906-5728

Omaha's Henry Doorly Zoo
3701 South 10th Street
Omaha, NE, 68107-2200

The Phoenix Zoo
455 N. Galvin Parkway
Phoenix, AZ, 85008-3431

National Zoological Park's Conservation
and Research Center
1500 Remount Road
Front Royal, VA, 22630

Toronto Zoo
361 A. Old Finch,
Scarborough, Ontario
M1B 5K7

Louisville Zoological Garden
P.O. Box 37250
Louisville, KY, 40233-7250

**APPENDIX II** - Laboratories from which paraffin-embedded tissues were retrieved. Diagnostic laboratories involved in postmortem examinations of black-footed ferrets from the current captive population.

| Diagnostic laboratories | Number of pathology reports reviewed |
|---|---|
| Wyoming State Veterinary Laboratory [a] | 162 |
| Ontario Veterinary College [b] | 13 |
| APL Veterinary Laboratories [c] | 3 |
| Michigan State University [d] | 2 |
| St. Louis Zoological Park [e] | 2 |
| South Dakota State University [f] | 1 |
| National Zoological Park [g] | 1 |

[a] Wyoming State Veterinary Laboratory (WSVL), (Elizabeth S. Williams), University of Wyoming, 1174 Snowy Range Road, Laramie, WY, 822070

[b] Ontario Veterinary College (OVC), (I. K. Barker), Department of Pathobiology University of Guelph, Guelph, Ontario, N1G 2W1

[c] All Creatures Pathology Service (ACPS), (Chris A. Schiller), 13633 N. Cave Creek Rd. Phoenix, AZ, 85022

[d] Michigan State University (MSU), (Jon S. Patterson), College of Veterinary Medicine Lansing, MI, 48909

[e] St. Louis Zoological Park (STL), (Mary C. Duncan), 1 Government Drive, Forest Park, St. Louis, MO, 63110

[f] South Dakota State University (SDSU), (David H. Zeman), Animal Disease Research and Diagnostic Laboratory, Box 2175, Brookings, SD 57007-1396

[g] National Zoological Park (NZP), (Richard J. Montali), Department of Pathology Washington, DC, 20008

**APPENDIX III** - Description of the variables collected from the studbook, the postmortem reports, and the clinical files. Variables marked with an asterisk (*) were calculated.

Animal identification and material inventory:

**SB#**: Studbook number.

**name**: House name.

**zoo#**: Identification numbers from different institutions.

**pm report**: 1: Postmortem report retrieved; 0: No postmortem examination done, or postmortem report not available.

**lab**: Name of the diagnostic laboratory where the postmortem examination was conducted.

**blocks**: 1: Paraffin block retrieved; 0: No paraffin block retrieved.

**slides#**: Postmortem number.

**instudy**: 1: Animal included in the study group; 0: Animal not included in the study group.

Biologic parameters:

**sex**: 1: male; 0: female; ".": undetermined.

**birth**: Date of birth (DD-MMM-YY).

**remove**: Date of death or of release (DD-MMM-YY).

**age***: Age in years.

**APPENDIX III - cont.**

**inbreed**: Wright inbreeding coefficient (Wright, 1922).

**dead**: 1: animal dead; 0: animal still alive, or released.

**dead>1***: 1: animal dead, but survived for at least 1 yr; 0: animal still alive, died before one year of age, or was released.

Familial parameters:

**sire**: Studbook number of the ferret's sire.

**dam**: Studbook number of the ferret's dam.

**#kids***: Total number of offspring in the study population.

**#litter***: Total number of litters in the study population.

**offsp( )***: Proportion of offspring in the study group that were affected by the event of interest in parenthesis.

**halfsib( )***: Fraction of siblings (common sire or dam) in the study group that were affected by the event of interest in parenthesis.

**fullsib( )***: Fraction of full siblings (common sire and dam) in the study group that were affected by the event of interest in parenthesis.

**parent( )***: Fraction of parents (sire and dam) in the study group that were affected by the event of interest in parenthesis.

**firstrel( )***: Fraction of first relative (either parents, siblings, or offspring) in the study group that were affected by the neoplasm in parenthesis.

**APPENDIX III - cont.**

Female reproductive parameters:

**firspar***: Age at first litter.

**lastpar***: Age at last litter.

**lacstart***: Estimate number of started lactations. Data estimated from survival of offspring; a lactation was considered started if at least one kit survived for five days.

**laccomp***: Estimate number of lactations completed. Data estimated from survival of offspring; a lactation was considered completed if at least one kit survived for 25 days.

**lacta***: Estimate number of lactations. Indicate the number of complete lactations plus the fraction of incomplete lactations (number of days during which at least one kit survived divided by 25 days). Data estimated from survival of offspring.

Pathology parameters:

**death**: Name of the condition that was believed to be the cause of death.

**cysts**: Presence (1) or absence (0) of intrahepatic biliary cysts.

**bile_cysta**: Presence (1) or absence (0) of biliary cystadenoma.

**bile_carcino**: Presence (1) or absence (0) of biliary cystadenocarcinoma.

**bile_tumour**: Presence (1) or absence (0) of a biliary neoplasm (cystadenocarcinoma or cystadenoma).

**renal**: Presence (1) or absence (0) of renal tubular neoplasm.

**APPENDIX III - cont.**

**sweat_adeno**: Presence (1) or absence (0) of adenoma of the sweat gland.

**sweat_carcino**: Presence (1) or absence (0) of adenocarcinoma of the sweat gland.

**sweat**: Presence (1) or absence (0) of a neoplasm of the sweat gland.

**apo_anal**: Presence (1) or absence (0) of adenocarcinoma of the apocrine gland of the anal sacs.

**mamm_adeno**: Presence (1) or absence (0) of adenoma of the mammary gland.

**mamm_carcino**: Presence (1) or absence (0) of adenocarcinoma of the mammary gland.

**mamm**: Presence (1) or absence (0) of a neoplasms of the mammary gland.

**prepu_adeno**: Presence (1) or absence (0) of adenoma of the apocrine preputial gland.

**prepu_carcino**: Presence (1) or absence (0) of adenocarcinoma of the apocrine preputial gland.

**prepu**: Presence (1) or absence (0) of a neoplasm of the apocrine preputial gland.

**scc**: Presence (1) or absence (0) of squamous cell carcinoma.

**car_anal**: Presence (1) or absence (0) of carcinoma of the anal sacs.

**sebac**: Presence (1) or absence (0) of adenoma of the sebaceous gland.

**basocell**: Presence (1) or absence (0) of basocellular tumour.

**epi_cyst**: Presence (1) or absence (0) of epidermal cyst.

**melano**: Presence (1) or absence (0) of ocular melanoma.

**APPENDIX III - cont.**

**haemangio:** Presence (1) or absence (0) of haemangioma.

**haem_sarco**: Presence (1) or absence (0) of haemangiosarcoma.

**vascular**: Presence (1) or absence (0) of haemangioma or haemangiosarcoma.

**fibroma**: Presence (1) or absence (0) of fibroma.

**spind_cell**: Presence (1) or absence (0) of spindle cell tumour of the soft tissue.

**olfac_neuro**: Presence (1) or absence (0) of olfactory neuroblastoma.

**uter_leio**: Presence (1) or absence (0) of uterine leiomyoma.

**nasal_car**: Presence (1) or absence (0) of nasal carcinoma.

**seminoma**: Presence (1) or absence (0) of seminoma.

**inters_cell**: Presence (1) or absence (0) of interstitial cell tumour.

**transit_cell**: Presence (1) or absence (0) of transitional cell carcinoma.

**ganglioneuro**: Presence (1) or absence (0) of ganglioneuroblastoma.

**undeter**: Presence (1) or absence (0) of other neoplasms of undetermined origin.

**apocrin**: Presence (1) or absence (0) of at least one apocrine neoplasm.

**epithel**: Presence (1) or absence (0) of at least one epithelial neoplasm.

**tumour**: Presence (1) or absence (0) of at least one neoplasm.

**invasive**: Presence (1) or absence (0) of at least one invasive neoplasm.

**nbtumour**: Number of neoplasms per ferret.

**APPENDIX III - cont.**

Management parameters:

**institutions***: Proportion of life spent at each institution (from 0 to 1 for each institution).

**colo_sprg**: Cheyenne Mountain Zoological Park.

**louisvill**: Louisville Zoological Garden.

**nzp**: National Zoological Park's Conservation and Research Center.

**omaha**: Omaha's Henry Doorly Zoo.

**phoenix**: The Phoenix Zoo.

**sybille**: USFWS National Black-Footed Ferret Conservation Center.

**toronto**: Toronto Zoo.

**other**: All the other institutions.

**release**: 1: animal has been released; 0: animal has not been released.

**APPENDIX IV** - Histological details of the different neoplasms encountered in black-footed ferrets. Shaded areas represent medians.

**A) Biliary cystadenocarcinomas**

| **Tissue invasion** | **Absent** | **Low** | **Moderate** | **Severe** |
|---|---|---|---|---|
| Cystadenocarcinoma (17)[1] | 5 | 0 | 12 | 0 |

| **Cellular anaplasia** | **Low** | **1** | **2** | **High** |
|---|---|---|---|---|
| Cystadenocarcinoma (17) | 1 | 2 | 12 | 4 |

| **Anisokaryosis (fold)** | **1** | **2** | **3** | **4** | **5** | **6** |
|---|---|---|---|---|---|---|
| Cystadenocarcinoma (17) | 0 | 2 | 4 | 8 | 3 | 0 |

| **Mitotic index** | **0** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10 +** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cystadenocarcinoma (17) | 0 | 7 | 6 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |

| **Other features** | **Osseous metaplasia** | **Scirrhous reaction** | **Central necrosis** | **Metastasis** |
|---|---|---|---|---|
| Cystadenocarcinoma (17) | 6 | 14 | 12 | 6 |

[1] Total number of cases examined.

**APPENDIX IV - cont.**

**B) Renal tubular neoplasms**

| **Tissue invasion** | **Absent** | **Low** | **Moderate** | **Severe** |
|---|---|---|---|---|
| Renal tubular neo. (36)[1] | 2 | 8 | 18 | 8 |

| **Cellular anaplasia** | **Low** | **1** | **2** | **High** |
|---|---|---|---|---|
| Renal tubular neo. (36) | 2 | 30 | 4 | 0 |

| **Anisokaryosis (fold)** | **1** | **2** | **3** | **4** | **5** | **6** |
|---|---|---|---|---|---|---|
| Renal tubular neo. (36) | 1 | 4 | 21 | 9 | 1 | 0 |

| **Mitotic index** | **0** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10 +** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Renal tubular neo. (36) | 7 | 20 | 3 | 5 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |

| **Other features** | **Osseous metaplasia** | **Scirrhous reaction** | **Central necrosis** | **Metastasis** |
|---|---|---|---|---|
| Renal tubular neo. (36) | 29 | 28 | 9 | 1 |

[1] Total number of cases examined.

**APPENDIX IV - cont.**

**C) Neoplasms of the sweat gland**

| Tissue invasion | Absent | Low | Moderate | Severe |
|---|---|---|---|---|
| Adenoma (12)[1] | 12 | 0 | 0 | 0 |
| Adenocarcinoma (14) | 4 | 6 | 2 | 2 |

| Cellular anaplasia | Low | 1 | 2 | High |
|---|---|---|---|---|
| Adenoma (12) | 6 | 6 | 0 | 0 |
| Adenocarcinoma (14) | 1 | 6 | 5 | 2 |

| Anisokaryosis (fold) | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Adenoma (12) | 0 | 6 | 4 | 1 | 1 | 0 |
| Adenocarcinoma (14) | 0 | 4 | 3 | 3 | 3 | 1 |

| Mitotic index | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 + |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Adenoma (12) | 8 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Adenocarcinoma (14) | 0 | 7 | 3 | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |

| Other features | Osseous metaplasia | Scirrhous reaction | Central necrosis | Metastasis |
|---|---|---|---|---|
| Adenoma (12) | 0 | 0 | 0 | 0 |
| Adenocarcinoma (14) | 0 | 9 | 7 | 2 |

[1] Total number of cases examined.

**APPENDIX IV - cont.**

**D) Neoplasms of the mammary gland**

| **Tissue invasion** | **Absent** | **Low** | **Moderate** | **Severe** |
|---|---|---|---|---|
| Adenoma (4)[1] | 4 | 0 | 0 | 0 |
| Adenocarcinoma (7) | 2 | 1 | 2 | 2 |
| Carcinosarcoma (2) | 0 | 0 | 0 | 2 |

| **Cellular anaplasia** | **Low** | **1** | **2** | **High** |
|---|---|---|---|---|
| Adenoma (4) | 3 | 1 | 0 | 0 |
| Adenocarcinoma (7) | 1 | 1 | 5 | 0 |
| Carcinosarcoma (2) | 0 | 0 | 1 | 1 |

| **Anisokaryosis (fold)** | **1** | **2** | **3** | **4** | **5** | **6** |
|---|---|---|---|---|---|---|
| Adenoma (4) | 0 | 3 | 1 | 0 | 0 | 0 |
| Adenocarcinoma (7) | 0 | 2 | 2 | 3 | 0 | 0 |
| Carcinosarcoma (2) | 0 | 0 | 0 | 1 | 1 | 0 |

| **Mitotic index** | **0** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10 +** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Adenoma (4) | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Adenocarcinoma (7) | 0 | 2 | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Carcinosarcoma (2) | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |

**APPENDIX IV - cont.**

**D) Neoplasms of the mammary gland (cont.)**

| Other features | Osseous metaplasia | Scirrhous reaction | Central necrosis | Metastasis |
|---|---|---|---|---|
| Adenoma (4) | 0 | 1 | 0 | 0 |
| Adenocarcinoma (7) | 0 | 3 | 2 | 1 |
| Carcinosarcoma (2) | 1 | 1 | 2 | 1 |

[1] Total number of cases examined.

**E) Epidermal cysts and squamous cell carcinomas (SCC)**

| Tissue invasion | Absent | Low | Moderate | Severe |
|---|---|---|---|---|
| Epidermal cyst (2) | 2 | 0 | 0 | 0 |
| Oral SCC (5) | 0 | 0 | 0 | 5 |
| Cutaneous SCC (1) | 0 | 0 | 0 | 1 |
| Anal sac SCC (2) | 0 | 0 | 0 | 2 |

| Cellular anaplasia | Low | 1 | 2 | High |
|---|---|---|---|---|
| Epidermal cyst (2) | 2 | 0 | 0 | 0 |
| Oral SCC (5) | 0 | 0 | 4 | 1 |
| Cutaneous SCC (1) | 0 | 1 | 0 | 0 |
| Anal sac SCC (2) | 0 | 0 | 1 | 1 |

**APPENDIX IV - cont.**

**E) Epidermal cysts and squamous cell carcinomas (SCC) (cont.)**

| **Anisokaryosis (fold)** | **1** | **2** | **3** | **4** | **5** | **6** |
|---|---|---|---|---|---|---|
| Epidermal cyst (2) | 0 | 2 | 0 | 0 | 0 | 0 |
| Oral SCC (5) | 0 | 0 | 1 | 3 | 1 | 0 |
| Cutaneous SCC (1) | 0 | 0 | 0 | 1 | 0 | 0 |
| Anal sac SCC (2) | 0 | 0 | 0 | 1 | 1 | 0 |

| **Mitotic index** | **0** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10 +** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Epidermal cyst (2) | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Oral SCC (5) | 0 | 0 | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cutaneous SCC (1) | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Anal sac SCC (2) | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |

| **Other features** | **Osseous metaplasia** | **Scirrhous reaction** | **Central necrosis** | **Metastasis** |
|---|---|---|---|---|
| Epidermal cyst (2) | 0 | 0 | 0 | 0 |
| Oral SCC (5) | 0 | 5 | 4 | 1 |
| Cutaneous SCC (1) | 0 | 1 | 1 | 0 |
| Anal sac SCC (2) | 0 | 2 | 0 | 0 |

[1] Total number of cases examined.

**APPENDIX IV - cont.**

**F) Adenocarcinomas of the apocrine gland of the anal sacs**

| **Tissue invasion** | **Absent** | **Low** | **Moderate** | **Severe** |
|---|---|---|---|---|
| Adenocarcinoma (14)[1] | 1 | 1 | 1 | 11 |

| **Cellular anaplasia** | **Low** | **1** | **2** | **High** |
|---|---|---|---|---|
| Adenocarcinoma (14) | 0 | 2 | 7 | 5 |

| **Anisokaryosis (fold)** | **1** | **2** | **3** | **4** | **5** | **6** |
|---|---|---|---|---|---|---|
| Adenocarcinoma (14) | 1 | 1 | 3 | 5 | 2 | 2 |

| **Mitotic index** | **0** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10 +** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Adenocarcinoma (14) | 0 | 1 | 4 | 2 | 1 | 1 | 1 | 1 | 0 | 0 | 3 |

| **Other features** | **Osseous metaplasia** | **Scirrhous reaction** | **Central necrosis** | **Metastasis** |
|---|---|---|---|---|
| Adenocarcinoma (14) | 2 | 13 | 7 | 9 |

[1] Total number of cases examined.

**APPENDIX IV - cont.**

## G) Adenocarcinomas of the preputial apocrine gland

| Tissue invasion | Absent | Low | Moderate | Severe |
|---|---|---|---|---|
| Adenoma (3)[1] | 3 | 0 | 0 | 0 |
| Adenocarcinoma (3) | 0 | 2 | 0 | 1 |

| Cellular anaplasia | Low | 1 | 2 | High |
|---|---|---|---|---|
| Adenoma (3) | 2 | 1 | 0 | 0 |
| Adenocarcinoma (3) | 0 | 1 | 1 | 1 |

| Anisokaryosis (fold) | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Adenoma (3) | 0 | 3 | 0 | 0 | 0 | 0 |
| Adenocarcinoma (3) | 0 | 0 | 1 | 2 | 0 | 0 |

| Mitotic index | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 + |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Adenoma (3) | 1 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Adenocarcinoma (3) | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 |

| Other features | Osseous metaplasia | Scirrhous reaction | Central necrosis | Metastasis |
|---|---|---|---|---|
| Adenoma (3) | 0 | 0 | 0 | 0 |
| Adenocarcinoma (3) | 1 | 3 | 3 | 1 |

[1] Total number of cases examined.

**APPENDIX IV - cont.**

**H) Adenomas of the sebaceous gland and basal cell tumours**

| **Tissue invasion** | **Absent** | **Low** | **Moderate** | **Severe** |
|---|---|---|---|---|
| Sebaceous adenoma (5)[1] | 3 | 2 | 0 | 0 |
| Basal cells tumour (2) | 2 | 0 | 0 | 0 |

| **Cellular anaplasia** | **Low** | **1** | **2** | **High** |
|---|---|---|---|---|
| Sebaceous adenoma (5) | 5 | 0 | 0 | 0 |
| Basal cells tumour (2) | 1 | 1 | 0 | 0 |

| **Anisokaryosis (fold)** | **1** | **2** | **3** | **4** | **5** | **6** |
|---|---|---|---|---|---|---|
| Sebaceous adenoma (5) | 0 | 0 | 5 | 0 | 0 | 0 |
| Basal cells tumour (2) | 0 | 2 | 0 | 0 | 0 | 0 |

| **Mitotic index** | **0** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10 +** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sebaceous adenoma (5) | 1 | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Basal cells tumour (2) | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |

| **Other features** | **Osseous metaplasia** | **Scirrhous reaction** | **Central necrosis** | **Metastasis** |
|---|---|---|---|---|
| Sebaceous adenoma (5) | 0 | 1 | 5 | 0 |
| Basal cells tumour (2) | 0 | 2 | 0 | 0 |

[1] Total number of cases examined.

## APPENDIX IV - cont.

## I) Various neoplasms

| Tissue invasion | Absent | Low | Moderate | Severe |
|---|---|---|---|---|
| Ocular melanoma (1)[1] | 0 | 0 | 1 | 0 |
| Haemangioma (2) | 2 | 0 | 0 | 0 |
| Haemangiosarcoma (1) | 0 | 0 | 0 | 1 |
| Uterine leiomyoma (2) | 2 | 0 | 0 | 0 |
| Fibroma (1) | 0 | 0 | 0 | 1 |
| Spindle-cell neoplasm (1) | 0 | 0 | 0 | 1 |
| Olfac. neuroblastoma (2) | 0 | 0 | 0 | 2 |
| Ganglioneuroblastoma (1) | 0 | 0 | 1 | 0 |
| Seminoma (1) | 0 | 1 | 0 | 0 |
| Interstitial cell tumour (2) | 0 | 1 | 1 | 0 |
| Transitional cell carci. (1) | 0 | 0 | 0 | 1 |
| Nasal carcinoma (1) | 0 | 0 | 0 | 1 |

| Cellular anaplasia | Low | 1 | 2 | High |
|---|---|---|---|---|
| Ocular melanoma (1) | 0 | 0 | 1 | 0 |
| Haemangioma (2) | 2 | 0 | 0 | 0 |
| Haemangiosarcoma (1) | 0 | 0 | 0 | 1 |
| Uterine leiomyoma (2) | 1 | 1 | 0 | 0 |
| Fibroma (1) | 0 | 1 | 0 | 0 |
| Spindle-cell neoplasm (1) | 0 | 1 | 0 | 0 |
| Olfac. neuroblastoma (2) | 0 | 2 | 0 | 0 |
| Ganglioneuroblastoma (1) | 1 | 1 | 0 | 0 |
| Seminoma (1) | 0 | 1 | 0 | 0 |
| Interstitial cell tumour (2) | 2 | 0 | 0 | 0 |
| Transitional cell carci. (1) | 0 | 0 | 0 | 1 |
| Nasal carcinoma (1) | 0 | 0 | 1 | 0 |

## APPENDIX IV - cont.

### I) Various neoplasms (cont.)

| Anisokaryosis (fold) | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Ocular melanoma (1) | 0 | 0 | 0 | 1 | 0 | 0 |
| Haemangioma (2) | 2 | 0 | 0 | 0 | 0 | 0 |
| Haemangiosarcoma (1) | 0 | 0 | 0 | 0 | 1 | 0 |
| Uterine leiomyoma (2) | 0 | 1 | 0 | 1 | 0 | 0 |
| Fibroma (1) | 0 | 0 | 1 | 0 | 0 | 0 |
| Spindle-cell neoplasm (1) | 0 | 0 | 1 | 0 | 0 | 0 |
| Olfac. neuroblastoma (2) | 0 | 0 | 2 | 0 | 0 | 0 |
| Ganglioneuroblastoma (1) | 0 | 1 | 0 | 0 | 0 | 0 |
| Seminoma (1) | 0 | 0 | 1 | 0 | 0 | 0 |
| Interstitial cell tumour (2) | 0 | 2 | 0 | 0 | 0 | 0 |
| Transitional cell carci. (1) | 0 | 0 | 0 | 0 | 0 | 1 |
| Nasal carcinoma (1) | 0 | 0 | 1 | 0 | 0 | 0 |

| Mitotic index | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 + |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Ocular melanoma (1) | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Haemangioma (2) | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Haemangiosarcoma (1) | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Uterine leiomyoma (2) | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Fibroma (1) | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Spindle-cell neoplasm (1) | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Olfac. neuroblastoma (2) | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| Ganglioneuroblastoma (1) | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Seminoma (1) | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Interstitial cell tumour (2) | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Transitional cell carci. (1) | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Nasal carcinoma (1) | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

## APPENDIX IV - cont.

### I) Various neoplasms (cont.)

| Other features | Osseous metaplasia | Scirrhous reaction | Central necrosis | Metastasis |
|---|---|---|---|---|
| Ocular melanoma (1) | 0 | 1 | 0 | 0 |
| Haemangioma (2) | 0 | 2 | 0 | 1 |
| Haemangiosarcoma (1) | 0 | 0 | 1 | 0 |
| Uterine leiomyoma (2) | 0 | 0 | 1 | 0 |
| Fibroma (1) | 0 | 0 | 0 | 0 |
| Spindle-cell neoplasm (1) | 0 | 1 | 1 | 0 |
| Olfac. neuroblastoma (2) | 0 | 2 | 2 | 0 |
| Ganglioneuroblastoma (1) | 0 | 1 | 0 | 0 |
| Seminoma (1) | 0 | 0 | 0 | 0 |
| Interstitial cell tumour (2) | 0 | 0 | 0 | 0 |
| Transitional cell carci. (1) | 0 | 0 | 0 | 0 |
| Nasal carcinoma (1) | 0 | 0 | 0 | 0 |

[1] Total number of cases examined.

**APPENDIX V** - Statistical significance (p-values) of association between potential risk factors and events of interest (neoplasia). Variables with p-values ≤ 0.25 will be tested in the logistic regression model.

| Test | Variables | Events of interest | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | cysts | tumour | epithel | apocrin | bile tumour | renal | sweat | apo anal | mamm[1] |
| Chisq[2] | Sex | 0.692 | 0.788 | 0.929 | 0.533 | 0.633 | 0.405 | 0.07 | 0.008 | - |
| | parent( ) | 0.957 | 0.122 | 0.243 | 0.426 | 0.259 | 0.798 | 0.475 | 1 | 0.374 |
| T-test[3] | birth | 0 .0001 | 0.0001 | 0.0001 | 0.0036 | 0.0001 | 0.0004 | 0.02 | 0.94 | 0.005 |
| | remove | 0.0001 | 0.003 | 0.0001 | 0.0005 | 0.049 | 0.016 | 0.002 | 0.0002 | 0.357 |
| | age | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| | inbreed | 0.0041 | 0.0007 | 0.0008 | 0.122 | 0.024 | 0.19 | 0.326 | 0.359 | 0.173 |
| | halfsib( ) | 0.034 | 0.0014 | 0.042 | 0.501 | 0.222 | 0.006 | 0.473 | 0.002 | 0.92 |
| | firstrel ( ) | 0.102 | 0.019 | 0.035 | 0.011 | 0.344 | 0.565 | 0.68 | 0.769 | 0.381 |
| | #kids | | | | | | | | | 0.06 |
| | #litter | | | | | | | | | 0.049 |
| | firspar | | | | | | | | | 0.992 |
| | lastpar | | | | | | | | | 0.385 |
| | lacta | | | | | | | | | 0.061 |

**APPENDIX V - cont.**

| Test | Variables | Events of interest | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | cysts | tumour | epithel | apocrin | bile tumour | renal | sweat | apo anal | mamm[1] |
| Wilcoxon[4] | toronto | 0.547 | 0.024 | 0.026 | 0.0001 | 0.471 | 0.035 | 0.001 | 0.015 | 0.426 |
| | sybille | 0.038 | 0.063 | 0.246 | 0.364 | 0.769 | 0.008 | 0.769 | 0.678 | 1 |
| | omaha | 0.066 | 0.662 | 0.706 | 0.721 | 0.224 | 0.85 | 0.383 | 0.363 | 0.721 |
| | nzp | 0.599 | 0.491 | 0.702 | 0.839 | 0.577 | 0.171 | 0.787 | 0.109 | 0.67 |
| | louisvill | 0.013 | 0.003 | 0.017 | 0.061 | 0.532 | 0.025 | 0.2 | 0.367 | 0.594 |
| | colo_sprg | 1 | 0.304 | 0.503 | 0.579 | 0.389 | 0.686 | 0.207 | 1 | 1 |
| | phoenix | 1 | 0.332 | 0.056 | 0.579 | 0.584 | 0.579 | 0.584 | 1 | 1 |
| | other | 0.775 | 0.366 | 0.181 | 0.563 | 0.532 | 0.025 | 0.532 | 0.629 | 1 |
| | fullsib( ) | 0.137 | 0.001 | 0.203 | 0.773 | 0.146 | 0.093 | 0.055 | 0.488 | 0.804 |
| | offsp( ) | 0.331 | 0.273 | 0.2 | 0.031 | 0.986 | 0.78 | 0.653 | 0.158 | 0.926 |

For definitions of the abbreviations see Appendix III

[1] Analysis for mammary tumours (mamm) done on subsamples of the study group composed of all the females.

[2] Chi-square test or Fisher's exact test.

[3] Two-sample t-test, for variables with normal distribution.

[4] Wilcoxon rank sum test, for variables with non-normal distribution.

**APPENDIX VI** - Biological and familial parameters of black-footed ferrets from the study group (n = 184). Empty spaces indicate that the information was not available.

| SB# | name | sex | sire | dam | age | inbreed | parity | | | | lactation | | sibling | | offsp( ) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | (year) | | #kids | #litter | firspar | lastpar | start | comp | halfsib( ) | fullsib( ) | |
| 6 | molly | F | | | 7.94 | | 1 | 0 | | | 1 | 1 | | | 0 |
| 10 | willa | F | | | | | 1 | 0 | | | 1 | 1 | | | 1 |
| 11 | emma | F | | | | | 2 | 1 | | | 2 | 2 | | | 0 |
| 12 | annie | F | | | | | 2 | 2 | | | 2 | 1.28 | | | 1 |
| 13 | dexter | M | 9 | 8 | 8.60 | | 21 | 13 | na[1] | na | na | na | 1 | 0 | 25 |
| 14 | cody | M | 9 | 10 | 6.25 | | 0 | 0 | na | na | na | na | 1 | 0 | 0 |
| 16 | dean | M | | | 5.50 | | 22 | 8 | na | na | na | na | | | 26 |
| 17 | jenny | F | | | 5.51 | | 11 | 4 | | 2.95 | 3 | 3 | | | 7 |
| 18 | scarface | M | | | | | 9 | 4 | na | na | na | na | | | 21 |
| 19 | becky | F | 18 | 17 | 5.97 | | 17 | 5 | 1.01 | 5.95 | 3 | 3 | 24 | 2 | 9 |
| 20 | rocky | M | 18 | 17 | | | 9 | 3 | na | na | na | na | 24 | 2 | 12 |
| 21 | sundance | M | 18 | 17 | | | 9 | 3 | na | na | na | na | 24 | 2 | 12 |
| 22 | mom | F | | | 7.75 | | 12 | 3 | | 2.97 | 3 | 3 | | | 10 |
| 23 | cutlip | M | 18 | 22 | 7.64 | | 5 | 2 | na | na | na | na | 23 | 6 | 5 |
| 24 | collene | F | 18 | 22 | 6.88 | | 6 | 2 | 1.96 | 2.86 | 2 | 2 | 23 | 6 | 4 |
| 25 | rene | F | 18 | 22 | 7.73 | | 6 | 2 | 1.97 | 2.98 | 1 | 1 | 23 | 6 | 3 |
| 26 | jez | F | 18 | 22 | 9.34 | | 13 | 4 | 1.96 | 5.99 | 2 | 2 | 23 | 6 | 7 |
| 27 | amy | F | 18 | 22 | 7.60 | | 11 | 3 | 1.97 | 4.94 | 3 | 2.44 | 23 | 6 | 7 |
| 28 | fennis | M | 18 | 19 | 7.16 | 0.25 | 0 | 0 | na | na | na | na | 23 | 5 | 0 |

**APPENDIX VI - cont.**

| SB# | name | sex | sire | dam | age (year) | inbreed | parity | | | | lactation | | sibling | | offsp( ) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | #kids | #litter | firspar | lastpar | start | comp | halfsib( ) | fullsib( ) | |
| 29 | sully | M | 18 | 19 | 8.02 | 0.25 | 0 | 0 | na | na | na | na | 23 | 5 | 0 |
| 30 | la verne | F | 18 | 19 | 8.30 | 0.25 | 12 | 5 | 0.99 | 4.98 | 4 | 4 | 23 | 5 | 6 |
| 31 | hannah | F | 18 | 19 | 8.47 | 0.25 | 15 | 4 | 1.01 | 3.99 | 3 | 3 | 23 | 5 | 12 |
| 32 | meeteetse | F | 18 | 19 | 6.75 | 0.25 | 4 | 4 | 0.98 | 3.11 | 2 | 2 | 23 | 5 | 2 |
| 33 | sadie | F | 18 | 19 | 6.70 | 0.25 | 6 | 4 | 1.94 | 5.91 | 2 | 2 | 23 | 5 | 0 |
| 34 | shelly | F | 13 | 17 | 7.98 | | 11 | 5 | 0.93 | 4.92 | 4 | 4 | 27 | 3 | 5 |
| 35 | danny | M | 16 | 19 | 7.12 | | 6 | 2 | na | na | na | na | 31 | 2 | 0 |
| 36 | bubba | M | 16 | 19 | 4.35 | | 1 | 1 | na | na | na | na | 31 | 2 | 1 |
| 37 | drifter | M | 16 | 19 | 1.06 | | 1 | 1 | na | na | na | na | 31 | 2 | 2 |
| 39 | kenny | M | 13 | 22 | 7.72 | | 42 | 23 | na | na | na | na | 31 | 2 | 11 |
| 40 | sage | F | 13 | 22 | 8.58 | | 8 | 3 | 2.00 | 3.03 | 2 | 2 | 31 | 2 | 0 |
| 41 | pseudo | F | 13 | 22 | 7.62 | | 0 | 0 | | | 0 | 0 | 31 | 2 | 0 |
| 43 | debbie | F | 21 | 26 | 7.20 | 0.125 | 6 | 3 | 1.11 | 3.03 | 1 | 1 | 16 | 1 | 0 |
| 44 | cora | F | 21 | 26 | 8.12 | 0.125 | 14 | 4 | 1.07 | 4.98 | 3 | 3 | 16 | 1 | 2 |
| 47 | casper | M | 13 | 24 | 8.12 | | 5 | 3 | na | na | na | na | 25 | 2 | 3 |
| 48 | gloria | F | 13 | 24 | 7.65 | | 20 | 6 | 1.04 | 5.07 | 4 | 4 | 25 | 2 | 5 |
| 49 | kaycee | F | 13 | 24 | 5.42 | | 11 | 4 | 1.03 | 3.97 | 4 | 4 | 25 | 2 | 8 |
| 51 | barker | M | 20 | 27 | 6.92 | 0.125 | 3 | 1 | na | na | na | na | 15 | 2 | 3 |
| 52 | willis | M | 20 | 27 | 5.84 | 0.125 | 13 | 6 | na | na | na | na | 15 | 2 | 1 |
| 53 | rebel | M | 20 | 27 | 7.78 | 0.125 | 0 | 0 | na | na | na | na | 15 | 2 | 0 |
| 56 | loretta | F | 13 | 17 | 5.47 | | 0 | 0 | | | 0 | 0 | 27 | 3 | 0 |
| 57 | rochelle | F | 13 | 17 | 9.05 | | 13 | 5 | 1.02 | 6.07 | 4 | 4 | 27 | 3 | 2 |
| 58 | gina | F | 13 | 17 | 8.71 | | 8 | 4 | 1.04 | 4.93 | 2 | 2 | 27 | 3 | 3 |

**APPENDIX VI - cont.**

| SB# | name | sex | sire | dam | age | inbreed | parity | | | | lactation | | sibling | | offsp( ) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | (year) | | #kids | #litter | firspar | lastpar | start | comp | halfsib( ) | fullsib( ) | |
| 60 | dwight | M | 16 | 25 | 7.39 | | 0 | 0 | na | na | na | na | 25 | 2 | 0 |
| 61 | byron | M | 16 | 25 | 8.11 | | 10 | 5 | na | na | na | na | 25 | 2 | 5 |
| 63 | shoshoni | F | 16 | 25 | 4.13 | | 15 | 5 | 1.91 | 4.12 | 3 | 3 | 25 | 2 | 4 |
| 66 | moneta | F | 16 | 32 | 8.15 | | 14 | 6 | 1.00 | 4.92 | 5 | 5 | 26 | 0 | 3 |
| 67 | bud | M | 13 | 30 | 7.73 | | 0 | 0 | na | na | na | na | 29 | 0 | 0 |
| 70 | festus | M | 18 | 34 | 5.91 | | 1 | 1 | na | na | na | na | 20 | 4 | 0 |
| 72 | gypsy | F | 18 | 34 | 8.48 | | 8 | 3 | 1.08 | 4.19 | 3 | 2.56 | 20 | 4 | 4 |
| 73 | isabell | F | 18 | 34 | 6.67 | | 7 | 4 | 1.01 | 4.00 | 4 | 4 | 20 | 4 | 0 |
| 75 | spook | F | 13 | 31 | 8.06 | | 7 | 2 | 2.03 | 2.96 | 2 | 2 | 32 | 3 | 1 |
| 76 | shirley | F | 13 | 31 | 7.34 | | 9 | 5 | 0.98 | 4.95 | 4 | 4 | 32 | 3 | 3 |
| 77 | frannie | F | 13 | 31 | 8.48 | | 11 | 4 | 0.97 | 4.92 | 3 | 3 | 32 | 3 | 5 |
| 78 | lb | F | 13 | 31 | 5.23 | | 0 | 0 | | | 0 | 0 | 32 | 3 | 0 |
| 79 | annieII | F | 13 | 12 | 5.66 | | 6 | 2 | 2.12 | 3.14 | 1 | 1 | 24 | 0 | 0 |
| 82 | shelby | F | 16 | 24 | 6.62 | | 9 | 2 | 1.06 | 2.11 | 2 | 2 | 28 | 0 | 3 |
| 83 | buck | M | 16 | 31 | 7.22 | | 0 | 0 | na | na | na | na | 31 | 5 | 0 |
| 84 | gasperII | M | 16 | 31 | 7.35 | | 7 | 3 | na | na | na | na | 31 | 5 | 1 |
| 85 | carl | M | 16 | 31 | 7.22 | | 0 | 0 | na | na | na | na | 31 | 5 | 0 |
| 86 | coy | M | 16 | 31 | 8.28 | | 0 | 0 | na | na | na | na | 31 | 5 | 0 |
| 87 | lisa | F | 16 | 31 | 7.92 | | 9 | 3 | 1.12 | 4.01 | 1 | 1 | 31 | 5 | 0 |
| 88 | marie | F | 16 | 31 | 4.01 | | 0 | 0 | | | 0 | 0 | 31 | 5 | 0 |
| 90 | clara | F | 18 | 34 | 7.21 | | 4 | 3 | 1.11 | 4.09 | 0 | 0 | 20 | 4 | 0 |
| 91 | irene | F | 18 | 34 | 6.56 | | 11 | 4 | 1.12 | 3.94 | 3 | 3 | 20 | 4 | 1 |
| 94 | bruno | M | 16 | 26 | 7.61 | | 38 | 19 | na | na | na | na | 27 | 4 | 5 |

**APPENDIX VI - cont.**

| SB# | name | sex | sire | dam | age | inbreed | parity | | | | lactation | | sibling | | offsp( ) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | (year) | | #kids | #litter | firspar | lastpar | start | comp | halfsib( ) | fullsib( ) | |
| 95 | codyII | M | 16 | 26 | 7.15 | | 0 | 0 | na | na | na | na | 27 | 4 | 0 |
| 96 | kristi | F | 16 | 26 | 5.26 | | 11 | 3 | 1.95 | 3.96 | 2 | 2 | 27 | 4 | 0 |
| 97 | jez | F | 16 | 26 | 6.41 | | 10 | 4 | 1.13 | 4.09 | 3 | 2.56 | 27 | 4 | 1 |
| 101 | meeteetse | M | 13 | 32 | 6.94 | | 0 | 0 | na | na | na | na | 25 | 0 | 0 |
| 104 | taurus | M | 18 | 22 | 7.62 | | 10 | 5 | na | na | na | na | 23 | 6 | 0 |
| 105 | astrid | F | 18 | 22 | 8.12 | | 16 | 3 | 1.93 | 3.90 | 3 | 3 | 23 | 6 | 3 |
| 108 | ariel | M | 16 | 30 | 5.13 | | 6 | 3 | na | na | na | na | 29 | 1 | 1 |
| 109 | caroline | F | 16 | 30 | 5.18 | | 14 | 4 | 1.01 | 3.95 | 4 | 4 | 29 | 1 | 3 |
| 110 | chimpi | M | 21 | 27 | 6.62 | 0.125 | 0 | 0 | na | na | na | na | 14 | 3 | 0 |
| 111 | elf | M | 21 | 27 | 7.52 | 0.125 | 5 | 3 | na | na | na | na | 14 | 3 | 0 |
| 112 | mark | M | 21 | 27 | 6.69 | 0.125 | 1 | 1 | na | na | na | na | 14 | 3 | 0 |
| 113 | lew | M | 21 | 27 | 7.11 | 0.125 | 4 | 2 | na | na | na | na | 14 | 3 | 0 |
| 116 | robby | M | 21 | 77 | 6.80 | 0.125 | 0 | 0 | na | na | na | na | 11 | 4 | 0 |
| 117 | tony | M | 21 | 77 | 7.10 | 0.125 | 16 | 5 | na | na | na | na | 11 | 4 | 1 |
| 118 | jeremy | M | 21 | 77 | 1.41 | 0.125 | 0 | 0 | na | na | na | na | 11 | 4 | 0 |
| 119 | red | M | 21 | 77 | 6.64 | 0.125 | 1 | 1 | na | na | na | na | 11 | 4 | 0 |
| 120 | marcy | F | 21 | 77 | 8.33 | 0.125 | 19 | 6 | 1.02 | 5.99 | 4 | 4 | 11 | 4 | 2 |
| 122 | cassidy | M | 23 | 57 | 7.52 | | 5 | 3 | na | na | na | na | 4 | 1 | 0 |
| 123 | mike | M | 23 | 57 | 4.05 | | 2 | 1 | na | na | na | na | 4 | 1 | 1 |
| 124 | chris | M | 13 | 66 | 7.49 | | 0 | 0 | na | na | na | na | 24 | 2 | 0 |
| 125 | stinky | M | 13 | 66 | 7.32 | | 0 | 0 | na | na | na | na | 24 | 2 | 0 |
| 126 | fannie | F | 13 | 66 | 5.48 | | 6 | 2 | 1.05 | 2.01 | 1 | 1 | 24 | 2 | 3 |
| 127 | scott | M | 20 | 49 | 3.09 | 0.0625 | 2 | 1 | na | na | na | na | 13 | 5 | 0 |

**APPENDIX VI - cont.**

| SB# | name | sex | sire | dam | age (year) | inbreed | parity | | | | lactation | | sibling | | offsp( ) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | #kids | #litter | firspar | lastpar | start | comp | halfsib( ) | fullsib( ) | |
| 128 | john | M | 20 | 49 | 5.63 | 0.0625 | 0 | 0 | na | na | na | na | 13 | 5 | 0 |
| 129 | sam | M | 20 | 49 | 7.56 | 0.0625 | 1 | 1 | na | na | na | na | 13 | 5 | 1 |
| 130 | tyler | M | 20 | 49 | 5.89 | 0.0625 | 1 | 1 | na | na | na | na | 13 | 5 | 1 |
| 131 | cindy | F | 20 | 49 | 6.00 | 0.0625 | 4 | 2 | 2.92 | 3.89 | 0 | 0 | 13 | 5 | 0 |
| 132 | keena | F | 20 | 49 | 1.84 | 0.0625 | 0 | 0 | | | 0 | 0 | 13 | 5 | 0 |
| 133 | gregg | M | 23 | 76 | 3.12 | 0.0937 | 0 | 0 | na | na | na | na | 4 | 2 | 0 |
| 134 | steve | M | 23 | 76 | 7.70 | 0.0937 | 0 | 0 | na | na | na | na | 4 | 2 | 0 |
| 135 | bb | F | 23 | 76 | 6.11 | 0.0937 | 12 | 3 | 1.12 | 4.04 | 1 | 1 | 4 | 2 | 1 |
| 136 | david | M | 69 | 48 | 2.61 | 0.125 | 0 | 0 | na | na | na | na | 11 | 1 | 0 |
| 137 | coccidio | M | 69 | 48 | 7.67 | 0.125 | 0 | 0 | na | na | na | na | 11 | 1 | 0 |
| 140 | kevin | M | 61 | 71 | 4.74 | 0.0625 | 9 | 5 | na | na | na | na | 5 | 2 | 0 |
| 141 | tammy | F | 61 | 71 | 6.81 | 0.0625 | 14 | 5 | 0.97 | 4.11 | 3 | 3 | 5 | 2 | 3 |
| 142 | teeter | F | 61 | 71 | 7.35 | 0.125 | 10 | 4 | 1.00 | 4.11 | 3 | 3 | 5 | 2 | 1 |
| 143 | willy | M | 69 | 58 | 6.62 | 0.125 | 16 | 8 | na | na | na | na | 8 | 2 | 4 |
| 144 | joey | M | 69 | 58 | 7.03 | 0.125 | 6 | 3 | na | na | na | na | 8 | 2 | 0 |
| 145 | tim | M | 69 | 58 | 2.89 | 0.125 | 0 | 0 | na | na | na | na | 8 | 2 | 0 |
| 149 | dan | M | 39 | 44 | 5.28 | 0.0625 | 0 | 0 | na | na | na | na | 11 | 0 | 0 |
| 154 | ross | M | 37 | 72 | 5.59 | 0.0937 | 0 | 0 | na | na | na | na | 3 | 1 | 0 |
| 155 | betsy | F | 37 | 72 | 5.89 | 0.0937 | 1 | 1 | 2.93 | 2.93 | 0 | 0 | 3 | 1 | 0 |
| 156 | star | M | 74 | 62 | 6.65 | 0.0468 | 2 | 1 | na | na | na | na | 6 | 3 | 2 |
| 157 | spangle | M | 74 | 62 | 4.61 | 0.0468 | 0 | 0 | na | na | na | na | 6 | 3 | 0 |
| 158 | banner | M | 74 | 62 | 5.74 | 0.0468 | 0 | 0 | na | na | na | na | 6 | 3 | 0 |
| 159 | derek | M | 16 | 26 | 6.45 | | 4 | 2 | na | na | na | na | 27 | 4 | 1 |

**APPENDIX VI - cont.**

| SB# | name | sex | sire | dam | age (year) | inbreed | parity | | | | lactation | | sibling | | offsp( ) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | #kids | #litter | firspar | lastpar | start | comp | halfsib( ) | fullsib( ) | |
| 167 | martin | M | 39 | 31 | 3.80 | | 0 | 0 | na | na | na | na | 21 | 0 | 0 |
| 171 | woodstock | M | 20 | 48 | 5.02 | 0.0625 | 0 | 0 | na | na | na | na | 13 | 2 | 0 |
| 172 | huey | M | 20 | 48 | 4.59 | 0.0625 | 10 | 4 | na | na | na | na | 13 | 2 | 1 |
| 173 | wendy | F | 20 | 48 | 7.95 | 0.0625 | 10 | 2 | 1.04 | 2.05 | 2 | 2 | 13 | 2 | 0 |
| 180 | lena | F | 13 | 82 | 3.69 | | 11 | 3 | 1.05 | 2.99 | 2 | 1.76 | 25 | 1 | 0 |
| 182 | attilia | F | 13 | 82 | 4.90 | | 0 | 0 | | | 0 | 0 | 25 | 1 | 0 |
| 187 | thomas | M | 39 | 30 | 6.17 | | 0 | 0 | na | na | na | na | 13 | 2 | 0 |
| 188 | bone | M | 39 | 30 | 6.80 | | 0 | 0 | na | na | na | na | 13 | 2 | 0 |
| 202 | don | M | 13 | 109 | 4.79 | | 16 | 9 | na | na | na | na | 26 | 0 | 2 |
| 210 | walter | M | 47 | 141 | 5.58 | 0.0937 | 0 | 0 | na | na | na | na | 4 | 0 | 0 |
| 212 | julie | F | 39 | 91 | 2.06 | 0.0625 | 12 | 2 | 0.99 | 2.00 | 1 | 1 | 10 | 0 | 0 |
| 213 | rick | M | 94 | 147 | 3.70 | 0.0625 | 0 | 0 | na | na | na | na | 4 | 1 | 0 |
| 215 | baby | M | 94 | 147 | 1.13 | 0.0625 | 0 | 0 | na | na | na | na | 4 | 1 | 0 |
| 219 | rhonda | F | 123 | 142 | 6.10 | 0.0937 | 9 | 3 | 0.99 | 2.05 | 2 | 2 | 0 | 0 | 3 |
| 221 | robert | M | 94 | 120 | 4.70 | 0.0546 | 0 | 0 | na | na | na | na | 4 | 1 | 0 |
| 222 | george | M | 94 | 120 | 5.78 | 0.0546 | 0 | 0 | na | na | na | na | 4 | 1 | 0 |
| 226 | lynn/uno | M | 52 | 75 | 6.04 | 0.1093 | 0 | 0 | na | na | na | na | 0 | 0 | 0 |
| 230 | bandit | M | 36 | 135 | 4.58 | 0.0781 | 0 | 0 | na | na | na | na | 0 | 0 | 0 |
| 233 | sarah | F | 156 | 72 | 5.16 | 0.1171 | 5 | 2 | 2.03 | 3.00 | 1 | 1 | 3 | 1 | 0 |
| 234 | sylvia | F | 156 | 72 | 6.11 | 0.1171 | 9 | 2 | 2.03 | 2.93 | 1 | 0.2 | 3 | 1 | 0 |
| 236 | brian | M | 51 | 126 | 6.67 | 0.0546 | 15 | 6 | na | na | na | na | 2 | 2 | 0 |
| 237 | starkey | M | 51 | 126 | 4.47 | 0.0546 | 0 | 0 | na | na | na | na | 2 | 2 | 0 |
| 238 | ranger | M | 51 | 126 | 4.93 | 0.0546 | 0 | 0 | na | na | na | na | 2 | 2 | 0 |

**APPENDIX VI - cont.**

| SB# | name | sex | sire | dam | age (year) | inbreed | parity | | | | lactation | | sibling | | offsp( ) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | #kids | #litter | firspar | lastpar | start | comp | halfsib( ) | fullsib( ) | |
| 241 | cibele | F | 74 | 81 | 2.60 | 0.0468 | 11 | 2 | 0.94 | 1.94 | 2 | 2 | 6 | 2 | 0 |
| 242 | heather | F | 74 | 81 | 4.93 | 0.0468 | 4 | 1 | 2.90 | 2.90 | 0 | 0 | 6 | 2 | 0 |
| 243 | jenny | F | 74 | 81 | 4.93 | 0.0468 | 8 | 2 | 2.01 | 3.00 | 1 | 1 | 6 | 2 | 0 |
| 245 | crazy larry | M | 74 | 62 | 6.21 | 0.0468 | 0 | 0 | na | na | na | na | 6 | 3 | 0 |
| 249 | deejay | M | 61 | 49 | 5.42 | 0.0625 | 0 | 0 | na | na | na | na | 10 | 1 | 0 |
| 250 | tinker | F | 61 | 49 | 4.96 | 0.0625 | 5 | 1 | 2.20 | 2.20 | 1 | 0.48 | 10 | 1 | 0 |
| 278 | steve | M | 13 | 178 | 4.60 | 0.125 | 0 | 0 | na | na | na | na | 24 | 1 | 0 |
| 281 | jackson | M | 13 | 178 | 6.35 | 0.125 | 4 | 3 | na | na | na | na | 24 | 1 | 0 |
| 283 | paco | M | 16 | 105 | 5.59 | | 24 | 11 | na | na | na | na | 25 | 2 | 2 |
| 285 | pepe | M | 16 | 105 | 1.79 | | 0 | 0 | na | na | na | na | 25 | 2 | 0 |
| 287 | chequita | F | 16 | 105 | 5.83 | | 6 | 1 | 1.11 | 1.11 | 1 | 1 | 25 | 2 | 0 |
| 304 | bo | M | 16 | 174 | 6.88 | | 1 | 1 | na | na | na | na | 25 | 1 | 0 |
| 305 | dot | M | 16 | 174 | 5.59 | | 11 | 5 | na | na | na | na | 25 | 1 | 1 |
| 328 | | F | 172 | 109 | 1.02 | 0.1718 | 0 | 0 | | | 0 | 0 | 2 | 0 | 0 |
| 339 | bryan | M | 117 | 82 | 4.62 | 0.0546 | 0 | 0 | na | na | na | na | 2 | 0 | 0 |
| 349 | keith | M | 39 | 30 | 5.63 | | 0 | 0 | na | na | na | na | 13 | 2 | 0 |
| 379 | carol | M | 47 | 219 | 6.28 | 0.1093 | 0 | 0 | na | na | na | na | 3 | 1 | 0 |
| 380 | merle | M | 47 | 219 | 5.19 | 0.1093 | 0 | 0 | na | na | na | na | 3 | 1 | 0 |
| 393 | booboo | M | 94 | 31 | 6.62 | 0.0937 | 0 | 0 | na | na | na | na | 15 | 0 | 0 |
| 403 | sundance | M | 39 | 195 | 5.37 | | 0 | 0 | na | na | na | na | 10 | 0 | 0 |
| 438 | monica | F | 84 | 42 | 6.32 | | 4 | 1 | 2.02 | 2.02 | 1 | 0.2 | 1 | 0 | 0 |
| 444 | emerson | M | 69 | 63 | 5.46 | 0.0625 | 0 | 0 | na | na | na | na | 8 | 3 | 0 |
| 445 | mary | F | 69 | 63 | 6.39 | 0.0625 | 0 | 0 | | | 0 | 0 | 8 | 3 | 0 |

**APPENDIX VI - cont.**

| SB# | name | sex | sire | dam | age (year) | inbreed | parity | | | | lactation | | sibling | | offsp( ) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | #kids | #litter | firspar | lastpar | start | comp | halfsib( ) | fullsib( ) | |
| 447 | angela | F | 69 | 63 | 3.95 | 0.0625 | 0 | 0 | | | 0 | 0 | 8 | 3 | 0 |
| 448 | stacy | F | 69 | 63 | 6.61 | 0.0625 | 5 | 2 | 2.05 | 2.95 | 0 | 0 | 8 | 3 | 0 |
| 449 | sandy | M | 143 | 150 | 2.15 | 0.1015 | 0 | 0 | na | na | na | na | 3 | 2 | 0 |
| 451 | jackie | F | 143 | 150 | 2.14 | 0.1015 | 0 | 0 | | | 0 | 0 | 3 | 2 | 0 |
| 454 | ellen | F | 143 | 150 | 5.70 | 0.1015 | 4 | 1 | 1.97 | 1.97 | 1 | 0.6 | 3 | 2 | 0 |
| 527 | jason | M | 39 | 109 | 1.75 | | 0 | 0 | na | na | na | na | 12 | 0 | 0 |
| 543 | thyme | F | 39 | 141 | 3.41 | 0.0625 | 9 | 2 | 1.99 | 2.91 | 2 | 2 | 11 | 1 | 0 |
| 544 | tiny | F | 39 | 141 | 3.41 | 0.0625 | 8 | 2 | 1.98 | 2.89 | 2 | 2 | 11 | 1 | 0 |
| 561 | tipi | F | 179 | 71 | 4.40 | 0.0937 | 15 | 3 | 1.05 | 3.02 | 2 | 2 | 4 | 0 | 0 |
| 621 | kent | M | 283 | 405 | 1.34 | 0.0625 | 0 | 0 | na | na | na | na | 3 | 1 | 0 |
| 622 | michael | M | 283 | 405 | 4.11 | 0.0625 | 0 | 0 | na | na | na | na | 3 | 1 | 0 |
| 629 | houdini | M | 39 | 219 | 1.04 | 0.0937 | 0 | 0 | na | na | na | na | 12 | 0 | 0 |
| 647 | roy | M | 143 | 97 | 2.38 | 0.0312 | 0 | 0 | na | na | na | na | 3 | 0 | 0 |
| 745 | ken | M | 202 | 160 | 3.61 | 0.0859 | 0 | 0 | na | na | na | na | 2 | 1 | 0 |
| 746 | barbie | F | 202 | 160 | 2.29 | 0.0859 | 6 | 2 | 1.00 | 2.06 | 2 | 2 | 2 | 1 | 0 |
| 783 | kate | F | 159 | 405 | 1.36 | 0.0625 | 4 | 1 | 1.06 | 1.06 | 1 | 1 | 3 | 0 | 0 |
| 788 | bertha | F | 21 | 163 | 1.75 | 0.125 | 0 | 0 | | | 0 | 0 | 11 | 0 | 0 |
| 808 | josephine | F | 280 | 340 | 3.13 | 0.082 | 0 | 0 | | | 0 | 0 | 0 | 0 | 0 |
| 816 | charles | M | 179 | 44 | 2.38 | 0.0468 | 3 | 2 | na | na | na | na | 2 | 0 | 0 |
| 916 | bouncer | M | 296 | 42 | 4.53 | 0.1093 | 3 | 1 | na | na | na | na | 1 | 0 | 0 |
| 1012 | vaca | F | | 478 | 2.65 | | 10 | 2 | 1.06 | 2.14 | 1 | 1 | 0 | 0 | 0 |
| 1057 | cathy | F | 767 | 405 | 1.15 | 0.0937 | 0 | 0 | | | 0 | 0 | 3 | 0 | 0 |

**APPENDIX VI - cont.**

| SB# | name | sex | sire | dam | age (year) | inbreed | parity | | | | lactation | | sibling | | offsp( ) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | #kids | #litter | firspar | lastpar | start | comp | halfsib( ) | fullsib( ) | |
| 1076 | anthony | M | 305 | 793 | 3.74 | 0.0976 | 6 | 4 | na | na | na | na | 0 | 0 | 0 |
| 1096 | buddy | M | 130 | 160 | 1.19 | 0.0625 | 0 | 0 | na | na | na | na | 2 | 0 | 0 |
| 1103 | | M | 108 | 631 | 1.00 | 0.0507 | 0 | 0 | na | na | na | na | 0 | 0 | 0 |
| 1222 | susanna | F | 129 | 664 | 2.15 | 0.0898 | 4 | 1 | 1.93 | 1.93 | 1 | 0.44 | 0 | 0 | 0 |
| 1336 | pixie | F | 1031 | 1018 | 1.64 | 0.0346 | 0 | 0 | | | 0 | 0 | 0 | 0 | 0 |

[1] na: Non applicable.

For definitions of the abbreviations see Appendix III.

**APPENDIX VII** - Pathologic findings in black-footed ferrets from the study group (n = 184).

| | | | | NEOPLASMS | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SB# | LAB | CAUSE OF DEATH | Cysts | Nbtumour | Bile tumour | Renal | Sweat | Apo anal | Mamm | Prepu | SCC | Sebac | Basocell | Epi cyst | Melanoma | Vascular | Fibroma | Olfac neuro | Uter leio | Nasal car | Seminoma | Inters cell | Transit cell | Ganglioneuro | Spind cell | Undeter |
| 6 | WSVL | Neoplasia | 1 | 2 | | | 1 | | 1 | | | | | | | | | | | | | | | | | |
| 10 | WSVL | Neoplasia | 1 | 1 | | | | | 0 | | | | | | | | | 1 | | | | | | | | |
| 11 | WSVL | Neoplasia | 1 | 5 | | | 1 | | | | 1 | 1 | | 1 | | 1 | | | | | | | | | | |
| 12 | WSVL | Undetermined | 1 | 2 | 1 | 1 | | | | | | | | | | | | | | | | | | | | |
| 13 | WSVL | Neoplasia | 1 | 3 | 1 | 1 | 1 | | | | | | | | | | | | | | | | | | | |
| 14 | WSVL | Meningitis | | 1 | | | | | | | | | | | | 1 | | | | | | | | | | |
| 16 | WSVL | Neoplasia | 1 | 3 | 1 | 1 | | | | 1 | | | | | | | | | | | | | | | | |
| 17 | WSVL | Possible septicemia | | | | | | | | | | | | | | | | | | | | | | | | |
| 18 | WSVL | Neoplasia | 1 | 4 | 1 | | 1 | | | | | | 1 | | | 1 | | | | | | | | | | |
| 19 | WSVL | Hemothorax | | | | | | | | | | | | | | | | | | | | | | | | |
| 20 | WSVL | Neoplasia | | 1 | | | | | | | | | | | | | | | | 1 | | | | | | |
| 21 | WSVL | Neoplasia | 1 | 1 | | | | | | | | | | | | | | | | | | | | | | 1 |
| 22 | WSVL | Undetermined | 1 | 2 | | | | | 1 | | | | | | | | | | 1 | | | | | | | |
| 23 | WSVL | Neoplasia | 1 | 1 | | 1 | | | | | | | | | | | | | | | | | | | | |
| 24 | WSVL | Neoplasia | 1 | 2 | | 1 | | | | | 1 | | | | | | | | | | | | | | | |
| 25 | WSVL | Undetermined | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| 26 | WSVL | Asphyxia, food | 1 | 1 | | 1 | | | | | | | | | | | | | | | | | | | | |

**APPENDIX VII - cont.**

| SB# | LAB | CAUSE OF DEATH | Cysts | NEOPLASMS | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Nbtumour | Bile tumour | Renal | Sweat | Apo anal | Mamm | Prepu | SCC | Sebac | Basocell | Epi cyst | Melanoma | Vascular | Fibroma | Olfac neuro | Uter leio | Nasal car | Seminona | Inters cell | Transit cell | Ganglioneuro | Spind cell | Undeter |
| 27 | WSVL | Neoplasia | | 2 | | 1 | | | 1 | | | | | | | | | | | | | | | | | |
| 28 | WSVL | Neoplasia | 1 | 2 | | | 1 | 1 | | | | | | | | | | | | | | | | | | |
| 29 | WSVL | Renal failure | 1 | 2 | 1 | 1 | | | | | | | | | | | | | | | | | | | | |
| 30 | WSVL | Neoplasia | 1 | 1 | 1 | | | | | | | | | | | | | | | | | | | | | |
| 31 | WSVL | Neoplasia | 1 | 2 | 1 | 1 | | | | | | | | | | | | | | | | | | | | |
| 32 | WSVL | Neoplasia | 1 | 1 | | | | | 1 | | | | | | | | | | | | | | | | | |
| 33 | WSVL | Renal failure | 1 | 1 | | 1 | | | | | | | | | | | | | | | | | | | | |
| 34 | WSVL | Pyothorax | 1 | 1 | | | | | | | | | | | | | 1 | | | | | | | | | |
| 35 | WSVL | Fibrosing interstitial pneumonia | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| 36 | WSVL | Undetermined | | | | | | | | | | | | | | | | | | | | | | | | |
| 37 | NZP | Undetermined | | | | | | | | | | | | | | | | | | | | | | | | |
| 39 | WSVL | Suppurative hepatitis/peritonitis | 1 | 1 | | | | | | | | 1 | | | | | | | | | | | | | | |
| 40 | WSVL | Neoplasia | 1 | 5 | 1 | 1 | 1 | | 1 | | | | | | | | | | 1 | | | | | | | |
| 41 | WSVL | Neoplasia | 1 | 2 | 1 | 1 | | | | | | | | | | | | | | | | | | | | |
| 43 | WSVL | Neoplasia | 1 | 1 | | | | | 1 | | | | | | | | | | | | | | | | | |
| 44 | WSVL | Neoplasia | 1 | 2 | 1 | | | | 1 | | | | | | | | | | | | | | | | | |
| 47 | WSVL | Suppurative hepatitis/peritonitis | 1 | 2 | | 1 | 1 | | | | | | | | | | | | | | | | | | | |
| 48 | WSVL | Neoplasia | 1 | 3 | 1 | | | 1 | 1 | | | | | | | | | | | | | | | | | |
| 49 | WSVL | Peritonitis | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| 51 | WSVL | Cardiac failure | 1 | 6 | 1 | 1 | 1 | 1 | | | | | | | | | | | | | 1 | 1 | | | | |

**APPENDIX VII - cont.**

| SB# | LAB | CAUSE OF DEATH | Cysts | NEOPLASMS | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Nbtumour | Bile tumour | Renal | Sweat | Apo anal | Mamm | Prepu | SCC | Sebac | Basocell | Epi cyst | Melanoma | Vascular | Fibroma | Olfac neuro | Uter leio | Nasal car | Seminoma | Inters cell | Transit cell | Ganglioneuro | Spind cell | Undeter |
| 52 | WSVL | Neoplasia | | 1 | | | | | | | 1 | | | | | | | | | | | | | | | |
| 53 | WSVL | Coccidiosis | 1 | 3 | 1 | | 1 | | | 1 | | | | | | | | | | | | | | | | |
| 56 | WSVL | Neoplasia | | 1 | | | | | | | 1 | | | | | | | | | | | | | | | |
| 57 | WSVL | Neoplasia | 1 | 2 | | 1 | 1 | | | | | | | | | | | | | | | | | | | |
| 58 | WSVL | Undetermined | 1 | 2 | 1 | | 1 | | | | | | | | | | | | | | | | | | | |
| 60 | WSVL | Neoplasia | 1 | 2 | 1 | 1 | | | | | | | | | | | | | | | | | | | | |
| 61 | WSVL | Neoplasia | 1 | 4 | 1 | 1 | | | | 1 | | | | 1 | | | | | | | | | | | | |
| 63 | WSVL | Undetermined | | | | | | | | | | | | | | | | | | | | | | | | |
| 66 | WSVL | Neoplasia | 1 | 1 | 1 | | | | | | | | | | | | | | | | | | | | | |
| 67 | WSVL | Suppurative hepatitis/peritonitis | 1 | 3 | 1 | | 1 | | | 1 | | | | | | | | | | | | | | | | |
| 70 | WSVL | Pyothorax | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| 72 | WSVL | Suppurative hepatitis/peritonitis | 1 | 1 | | 1 | | | | | | | | | | | | | | | | | | | | |
| 73 | WSVL | Neoplasia | 1 | 2 | | | 1 | | 1 | | | | | | | | | | | | | | | | | |
| 75 | WSVL | Undetermined | 1 | 1 | | | 1 | | | | | | | | | | | | | | | | | | | |
| 76 | WSVL | Fibrosing interstitial pneumonia | 1 | 2 | | | 1 | | | | | 1 | | | | | | | | | | | | | | |
| 77 | WSVL | Neoplasia | 1 | 2 | 1 | 1 | | | | | | | | | | | | | | | | | | | | |
| 78 | WSVL | Neoplasia | | 1 | | | | | | | | | | | | | | 1 | | | | | | | | |
| 79 | WSVL | Neoplasia | 1 | 1 | 1 | | | | | | | | | | | | | | | | | | | | | |
| 82 | WSVL | Renal failure | 1 | 1 | | 1 | | | | | | | | | | | | | | | | | | | | |
| 83 | WSVL | Neoplasia | 1 | 1 | | | | 1 | | | | | | | | | | | | | | | | | | |

**APPENDIX VII - cont.**

| SB# | LAB | CAUSE OF DEATH | Cysts | NEOPLASMS | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Nbtumour | Bile tumour | Renal | Sweat | Apo anal | Mamm | Prepu | SCC | Sebac | Basocell | Epi cyst | Melanoma | Vascular | Fibroma | Olfac neuro | Uter leio | Nasal car | Seminona | Inters cell | Transit cell | Ganglioneuro | Spind cell | Undeter |
| 84 | WSVL | Neoplasia | 1 | 3 | | | 1 | 1 | | | | | | | | | | | | | | | | | | 1 |
| 85 | WSVL | Post surgical hemorrhage | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| 86 | OVC | Neoplasia | 1 | 4 | | 1 | 1 | 1 | | | 1 | | | | | | | | | | | | | | | |
| 87 | WSVL | Neoplasia | 1 | 1 | | | | | 1 | | | | | | | | | | | | | | | | | |
| 88 | WSVL | Suppurative hepatitis/peritonitis | | | | | | | | | | | | | | | | | | | | | | | | |
| 90 | WSVL | Fibrosing interstitial pneumonia | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| 91 | WSVL | Esophageal puncture | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| 94 | WSVL | Neoplasia | 1 | 2 | 1 | | 1 | | | | | | | | | | | | | | | | | | | |
| 95 | WSVL | Neoplasia | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| 96 | WSVL | Undetermined | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| 97 | MSU | Undetermined | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| 101 | OVC | Biliary cysts | 1 | 2 | | | 1 | | | | | | | | 1 | | | | | | | | | | | |
| 104 | WSVL | Suppurative hepatitis/peritonitis | 1 | 1 | | | | | | | | | | | | | | | | | | 1 | | | | |
| 105 | WSVL | Suppurative hepatitis/peritonitis | 1 | 4 | 1 | 1 | 1 | | 1 | | | | | | | | | | | | | | | | | |
| 108 | WSVL | Neoplasia | 1 | 1 | | | | | | | | | | | | | | | | | | | | | | 1 |
| 109 | WSVL | Neoplasia | 1 | 2 | | 1 | | | | | 1 | | | | | | | | | | | | | | | |
| 110 | WSVL | Neoplasia | 1 | 4 | 1 | | 1 | | | 1 | | 1 | | | | | | | | | | | | | | |
| 111 | WSVL | Undetermined | 1 | 2 | | 1 | 1 | | | | | | | | | | | | | | | | | | | |
| 112 | ACPS | Neoplasia | 1 | 1 | 1 | | | | | | | | | | | | | | | | | | | | | |
| 113 | WSVL | Undetermined | 1 | | | | | | | | | | | | | | | | | | | | | | | |

**APPENDIX VII - cont.**

| | | | | NEOPLASMS | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SB# | LAB | CAUSE OF DEATH | Cysts | Nbtumour | Bile tumour | Renal | Sweat | Apo anal | Mamm | Prepu | SCC | Sebac | Basocell | Epi cyst | Melanoma | Vascular | Fibroma | Olfac neuro | Uter leio | Nasal car | Seminona | Inters cell | Transit cell | Ganglioneuro | Spind cell | Undeter |
| 116 | WSVL | Neoplasia | 1 | 3 | 1 | 1 | | 1 | | | | | | | | | | | | | | | | | | |
| 117 | WSVL | Suppurative hepatitis/peritonitis | 1 | 1 | 1 | | | | | | | | | | | | | | | | | | | | | |
| 118 | WSVL | Pyothorax | | | | | | | | | | | | | | | | | | | | | | | | |
| 119 | WSVL | Suppurative hepatitis/peritonitis | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| 120 | WSVL | Undetermined | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| 122 | WSVL | Metritis | 1 | 2 | 1 | 1 | | | | | | | | | | | | | | | | | | | | |
| 123 | WSVL | Meningitis | | | | | | | | | | | | | | | | | | | | | | | | |
| 124 | WSVL | Undetermined | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| 125 | STL | Undetermined | | 1 | | | | | | | | | | | | | | | | | | | | | 1 | |
| 126 | WSVL | Undetermined | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| 127 | WSVL | Toxoplasmosis | | | | | | | | | | | | | | | | | | | | | | | | |
| 128 | WSVL | Fibrosing interstitial pneumonia | | 1 | | | 1 | | | | | | | | | | | | | | | | | | | |
| 129 | ACPS | Neoplasia | 1 | 2 | 1 | 1 | | | | | | | | | | | | | | | | | | | | |
| 130 | WSVL | Fibrosing interstitial pneumonia | 1 | 1 | | 1 | | | | | | | | | | | | | | | | | | | | |
| 131 | WSVL | Fibrosing interstitial pneumonia | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| 132 | WSVL | Undetermined | | | | | | | | | | | | | | | | | | | | | | | | |
| 133 | WSVL | Neoplasia | 1 | 1 | 1 | | | | | | | | | | | | | | | | | | | | | |
| 134 | WSVL | Neoplasia | 1 | 2 | 1 | 1 | | | | | | | | | | | | | | | | | | | | |
| 135 | MSU | Septicemia | 1 | 1 | | 1 | | | | | | | | | | | | | | | | | | | | |
| 136 | WSVL | Bedding related | | | | | | | | | | | | | | | | | | | | | | | | |

**APPENDIX VII - cont.**

| SB# | LAB | CAUSE OF DEATH | Cysts | NEOPLASMS | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Nbtumour | Bile tumour | Renal | Sweat | Apo anal | Mamm | Prepu | SCC | Sebac | Basocell | Epi cyst | Melanoma | Vascular | Fibroma | Olfac neuro | Uter leio | Nasal car | Seminoma | Interstitial cell | Transit cell | Ganglioneuro | Spind cell | Undeter |
| 137 | WSVL | Undetermined | 1 | 1 | | | 1 | | | | | | | | | | | | | | | | | | | | |
| 140 | WSVL | Toxoplasmosis | | 0 | | | | | | | | | | | | | | | | | | | | | | | |
| 141 | WSVL | Pyothorax | 1 | 1 | | | | | 1 | | | | | | | | | | | | | | | | | | |
| 142 | SDSU | Undetermined | 1 | | | | | | | | | | | | | | | | | | | | | | | | |
| 143 | STL | Undetermined | | 3 | | | 1 | 1 | | | | | 1 | | | | | | | | | | | | | | |
| 144 | WSVL | Neoplasia | 1 | 1 | 1 | | | | | | | | | | | | | | | | | | | | | | |
| 145 | WSVL | Undetermined | | | | | | | | | | | | | | | | | | | | | | | | | |
| 149 | WSVL | Toxoplasmosis | 1 | | | | | | | | | | | | | | | | | | | | | | | | |
| 154 | WSVL | Neoplasia | | 2 | | | 1 | 1 | | | | | | | | | | | | | | | | | | | |
| 155 | WSVL | Toxoplasmosis | | | | | | | | | | | | | | | | | | | | | | | | | |
| 156 | WSVL | Neoplasia | 1 | 1 | | | | | | | | | | | | | | | | | | | 1 | | | | |
| 157 | WSVL | Toxoplasmosis | | | | | | | | | | | | | | | | | | | | | | | | | |
| 158 | WSVL | Ulcerative keratitis | | | | | | | | | | | | | | | | | | | | | | | | | |
| 159 | WSVL | Suppurative hepatitis/peritonitis | 1 | | | | | | | | | | | | | | | | | | | | | 1 | | | |
| 167 | WSVL | Neoplasia | | 1 | | | | | | | 1 | | | | | | | | | | | | | | | | |
| 171 | WSVL | Neoplasia | 1 | 1 | | 1 | | | | | | | | | | | | | | | | | | | | | |
| 172 | WSVL | Pyothorax | 1 | | | | | | | | | | | | | | | | | | | | | | | | |
| 173 | OVC | Neoplasia | | 1 | | 1 | 1 | | | | | | | | | | | | | | | | | | | | |
| 180 | WSVL | Pyothorax | | | | | | | | | | | | | | | | | | | | | | | | | |
| 182 | WSVL | Undetermined | | 1 | | | | | | | | | | | | | | | | | | | | | 1 | | |

**APPENDIX VII - cont.**

| SB# | LAB | CAUSE OF DEATH | Cysts | NEOPLASMS | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Nbtumour | Bile tumour | Renal | Sweat | Apo anal | Mamm | Prepu | SCC | Sebac | Basocell | Epi cyst | Melanoma | Vascular | Fibroma | Olfac neuro | Uter leio | Nasal car | Seminoma | Inters cell | Transit cell | Ganglioneuro | Spind cell | Undeter |
| 187 | WSVL | Ruptured bladder | 1 | 1 | 1 | | | | | | | | | | | | | | | | | | | | | |
| 188 | WSVL | Neoplasia | 1 | 2 | | | 1 | 1 | | | | | | | | | | | | | | | | | | |
| 202 | WSVL | Predation, ferret | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| 210 | WSVL | Suppurative hepatitis/peritonitis | 1 | 1 | | | | | | | | 1 | | | | | | | | | | | | | | |
| 212 | WSVL | Toxoplasmosis | | | | | | | | | | | | | | | | | | | | | | | | |
| 213 | WSVL | Toxoplasmosis | | | | | | | | | | | | | | | | | | | | | | | | |
| 215 | WSVL | Coccidiosis | | | | | | | | | | | | | | | | | | | | | | | | |
| 219 | WSVL | Cerebral cyst | 1 | 1 | | 1 | | | | | | | | | | | | | | | | | | | | |
| 221 | WSVL | Meningitis | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| 222 | WSVL | Neoplasia | 1 | 1 | | | | | | | 1 | | | | | | | | | | | | | | | |
| 226 | WSVL | Renal failure | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| 230 | WSVL | Neoplasia | 1 | 1 | 1 | | | | | | | | | | | | | | | | | | | | | |
| 233 | WSVL | Toxoplasmosis | | | | | | | | | | | | | | | | | | | | | | | | |
| 234 | WSVL | Toxoplasmosis | 1 | 1 | 1 | | | | | | | | | | | | | | | | | | | | | |
| 236 | WSVL | Meningoencephalitis | 1 | 1 | | | 1 | | | | | | | | | | | | | | | | | | | |
| 237 | WSVL | Toxoplasmosis | | | 0 | | | | | | | | | | | | | | | | | | | | | |
| 238 | WSVL | Toxoplasmosis | 1 | 1 | 1 | | | | | | | | | | | | | | | | | | | | | |
| 241 | WSVL | Undetermined | | | | | | | | | | | | | | | | | | | | | | | | |
| 242 | WSVL | Toxoplasmosis | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| 243 | WSVL | Toxoplasmosis | | | | | | | | | | | | | | | | | | | | | | | | |

**APPENDIX VII - cont.**

| | | | | NEOPLASMS | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SB# | LAB | CAUSE OF DEATH | Cysts | Nbtumour | Bile tumour | Renal | Sweat | Apo anal | Mamm | Prepu | SCC | Sebac | Basocell | Epi cyst | Melanoma | Vascular | Fibroma | Olfac neuro | Uter leio | Nasal car | Seminoma | Inters cell | Transit cell | Ganglioneuro | Spind cell | Undeter |
| 245 | OVC | Neoplasia | 1 | 3 | | | 1 | 1 | | 1 | | | | | | | | | | | | | | | | |
| 249 | WSVL | Fibrosing interstitial pneumonia | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| 250 | ACPS | Peritonitis due to uterine rupture | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| 278 | OVC | Neoplasia | | 1 | | | | | | | 1 | | | | | | | | | | | | | | | |
| 281 | OVC | Fibrosing interstitial pneumonia | 1 | 2 | | | 1 | | | | 1 | | | | | | | | | | | | | | | |
| 283 | WSVL | Plague (experimental) | 1 | 0 | | | | | | | | | | | | | | | | | | | | | | |
| 285 | WSVL | Plague | | | | | | | | | | | | | | | | | | | | | | | | |
| 287 | WSVL | Fibrosing interstitial pneumonia | 1 | 1 | | 1 | | | | | | | | | | | | | | | | | | | | |
| 304 | OVC | Undetermined | 1 | 3 | | 1 | 1 | 1 | | | | | | | | | | | | | | | | | | |
| 305 | WSVL | Bedding related | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| 328 | WSVL | Coccidiosis | | | | | | | | | | | | | | | | | | | | | | | | |
| 339 | OVC | Neoplasia | | 2 | | 1 | | 1 | | | | | | | | | | | | | | | | | | |
| 349 | WSVL | Suppurative hepatitis/peritonitis | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| 379 | WSVL | Neoplasia | 1 | 1 | | | | 1 | | | | | | | | | | | | | | | | | | |
| 380 | WSVL | Pyothorax | | 1 | | | 1 | | | | | | | | | | | | | | | | | | | |
| 393 | OVC | Neoplasia | 1 | 2 | | 1 | 1 | | | | | | | | | | | | | | | | | | | |
| 403 | WSVL | Toxoplasmosis | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| 438 | OVC | Neoplasia | 1 | 1 | | | | | 1 | | | | | | | | | | | | | | | | | |
| 444 | WSVL | Plague (experimental) | 1 | 0 | | | | 0 | 0 | | | | | | | | | | | | | | | | | |
| 445 | WSVL | Neoplasia | 1 | 2 | 1 | | | 1 | | | | | | | | | | | | | | | | | | |

**APPENDIX VII - cont.**

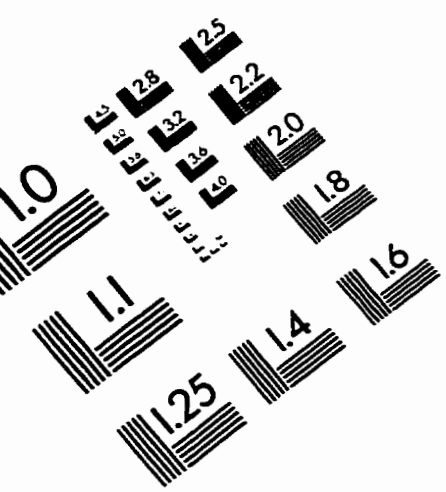
| | | | | NEOPLASMS | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SB# | LAB | CAUSE OF DEATH | Cysts | Nbtumour | Bile tumour | Renal | Sweat | Apo anal | Mamm | Prepu | SCC | Sebac | Basocell | Epi cyst | Melanoma | Vascular | Fibroma | Olfac neuro | Uter leio | Nasal car | Seminoma | Inters cell | Transit cell | Ganglioneuro | Spind cell | Undeter |
| 447 | WSVL | Cerebral cyst | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| 448 | OVC | Neoplasia | 1 | 3 | 1 | 1 | 1 | | | | | | | | | | | | | | | | | | | |
| 449 | WSVL | Coccidiosis | | | | | | | | | | | | | | | | | | | | | | | | |
| 451 | WSVL | Coccidiosis | | | | | | | | | | | | | | | | | | | | | | | | |
| 454 | WSVL | Neoplasia | 1 | 1 | | | | | | | | | | | | | | | | 1 | | | | | | |
| 527 | WSVL | Trauma tongue | | | | | | | | | | | | | | | | | | | | | | | | |
| 543 | WSVL | Predation, coyote | | | | | | | | | | | | | | | | | | | | | | | | |
| 544 | WSVL | Predation, coyote | | | | | | | | | | | | | | | | | | | | | | | | |
| 561 | WSVL | Undetermined | | | | | | | | | | | | | | | | | | | | | | | | |
| 621 | WSVL | Enteritis | | | | | | | | | | | | | | | | | | | | | | | | |
| 622 | WSVL | Biliary cyst | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| 629 | WSVL | Coccidiosis | | | | | | | | | | | | | | | | | | | | | | | | |
| 647 | WSVL | Cryptosporidiosis | | | | | | | | | | | | | | | | | | | | | | | | |
| 745 | WSVL | Plague (experimental) | | | | | | | | | | | | | | | | | | | | | | | | |
| 746 | WSVL | Myocarditis | | | | | | | | | | | | | | | | | | | | | | | | |
| 783 | WSVL | Undetermined | | | | | | | | | | | | | | | | | | | | | | | | |
| 788 | WSVL | Renal failure | | | | | | | | | | | | | | | | | | | | | | | | |
| 808 | WSVL | Asphyxia, food related | | | | | | | | | | | | | | | | | | | | | | | | |
| 816 | WSVL | Bacterial enteritis | | | | | | | | | | | | | | | | | | | | | | | | |
| 916 | OVC | Vegetative endocarditis | 1 | | | | | | | | | | | | | | | | | | | | | | | |

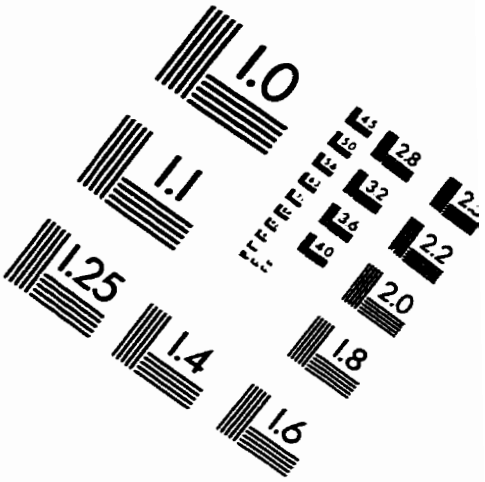
**APPENDIX VII - cont.**

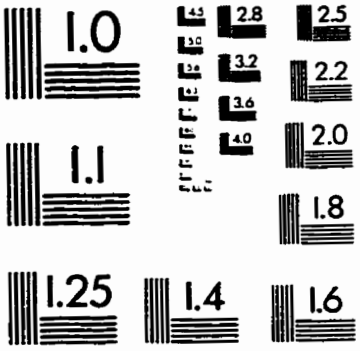
| SB# | LAB | CAUSE OF DEATH | Cysts | NEOPLASMS | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Nbtumour | Bile tumour | Renal | Sweat | Apo anal | Mamm | Prepu | SCC | Sebac | Basocell | Epi cyst | Melanoma | Vascular | Fibroma | Olfac neuro | Uter leio | Nasal car | Seminoma | Inters cell | Transit cell | Ganglioneuro | Spind cell | Undeter |
| 1012 | WSVL | Predation, ferret | | | | | | | | | | | | | | | | | | | | | | | | |
| 1057 | WSVL | Meningitis | | | | | | | | | | | | | | | | | | | | | | | | |
| 1076 | OVC | Renal failure | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| 1096 | WSVL | Coccidiosis | | | | | | | | | | | | | | | | | | | | | | | | |
| 1103 | WSVL | Acute fibrinous pneumonia | | | | | | | | | | | | | | | | | | | | | | | | |
| 1222 | WSVL | Coccidiosis | | | | | | | | | | | | | | | | | | | | | | | | |
| 1336 | WSVL | Predation, ferret | | | | | | | | | | | | | | | | | | | | | | | | |

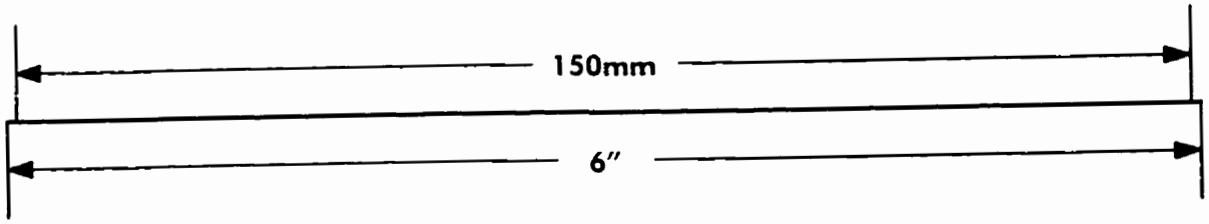
For definitions of abbreviations see Appendixes II, and III.

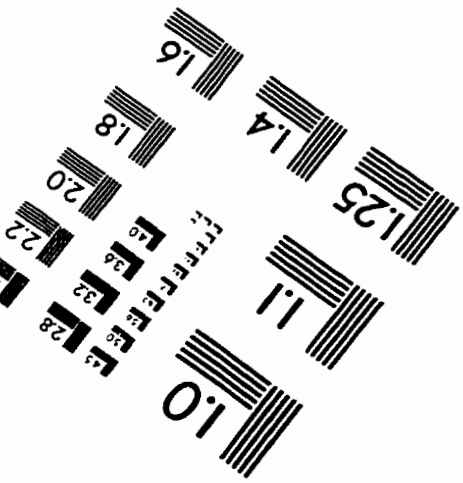
# IMAGE EVALUATION
# TEST TARGET (QA-3)

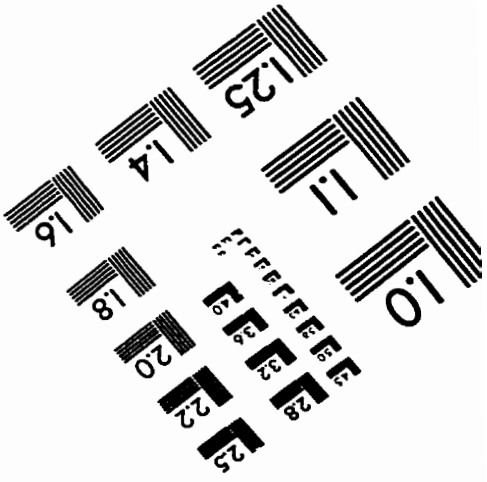
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