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Case Study

Pathology of Ground Squirrels in a Research Colony

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This paper presents a retrospective review of the postmortem findings in a colony of wild-caught ground squirrels used in medical research. The species included in this study were Richardson's ground squirrel *Urocitellus richardsonii*, Columbian ground squirrel *Urocitellus columbianus* and golden-mantled ground squirrel *Callospermophilus lateralis*. The pathologic findings in 160 ground squirrels from this colony demonstrated a wide variety of conditions, with chronic nephritis and hepatic adenomas being the most frequent overall. All animals with gross lesions of chronic interstitial nephritis had both glomerular and tubulointerstitial disease upon microscopic examination. As the first review of pathology in a research colony of ground squirrels. this study provides data for use in comparative studies about rodent diseases and important information for those who maintain such animals for research.

Abbreviations: U of A, University of Alberta

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Ground squirrels are commonly but incorrectly referred to as gophers and are terrestrial members of the Order Rodentia, family Sciuridae. True gophers are members of the family Geomyidae. In the Canadian province of Alberta, the 3 most common species of ground squirrels are Richardson ground squirrel (*Urocitellus richardsonii)*,[4,13] the Columbian ground squirrel (*Urocitellus columbianus)*,[5] and the golden-mantled ground squirrel (*Callospermophilus lateralis)*.[7] Thirteen lined (*Ictidomys tridecemlineatus) and* Franklin (*Poliocitellus franklinii)*[6] ground squirrels are also present but less numerous in Alberta.

The 3 species in this study vary in lifespan, litter size, population density,[5] annual mortality rate and timing of reproduction,[13] but have several common attributes. An overview of these ground squirrels in the wild is as follows. In a natural habitat, which includes open sagebrush, grasslands,[4] and mountain meadows, ground squirrels live in complex burrows on stable,[4] sandy, well-drained soils[4,5] at elevations from 1828 to over 3658 meters in the western 2-thirds of North America.[1] Litters are born in late April to early May and consist of 6 to 8 pups. Ground squirrels are social animals, with densities ranging from 5 to 60 per hectare. In the wild, mortality among male Richardson ground squirrels is as much as 80% per year, partly due to heavy predation.[14] Females have a slightly lower annual mortality rate and are considered to have reached old age at 3 to 4 y of age.[14] One study in southern Alberta found 5 and 6 y old females were present but only a single male that had survived to 4 y.[13] Ground squirrel burrows can damage to agricultural land because their holes are a hazard to livestock, their mounds can damage equipment, and burrowing can damage crops.[11] For these reasons, they are often considered a pest species. On the other hand, ground squirrels till the soil and can serve as a food source for predators.[11] As such, they are an integral part of the prairie and mountain ecosystem and food chain.

Ground squirrels are most often used to study hibernation and metabolism, as their basal metabolic rate varies according to environmental conditions. They have large amounts of brown fat and are deep seasonal hibernators.[3] Unlike most rodents, they are strictly diurnal with peak activities in the morning and late afternoon. Hibernation in ground squirrels consists of periods of torpor interspersed with brief thermogenic arousals that last less than 24 h. Torpor bouts increase in length with decreasing soil temperature. During periods of inactivity, body temperature drops to near ambient soil temperature, falling as low as 2 to 3 °C in late winter.[12] Despite the metabolic costs of thermogenesis and euthermy during intertorpor arousals, hibernating ground squirrels can achieve an overall energy saving over the hibernating season of 88% compared with euthermic animals.[19] Complex regulation of body temperature makes ground squirrels ideal subjects for studies of basal metabolism,[9] body composition during euthermic and hibernating states, and gene expression during hibernation.[8] Other research applications for which ground squirrels are useful include the study of liver neoplasia,[10,15,18] cholesterol gallstones,[17] obesity, and diabetes mellitus.[15]

Ground squirrels seldom produce fertile matings in captivity, and research colonies must be maintained by capture from the wild, posing biosecurity challenges for such colonies. Despite the need to capture of wild ground squirrels for research, these animals habituate easily and adapt well to captivity

Knowledge of the pathology of captive ground squirrels is important to investigators and veterinarians who maintain colonies for research purposes. Although wild ground squirrels are used as a laboratory animal in some research institutions, little information is available in the scientific literature about the naturally occurring diseases and pathology of ground

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squirrels, with the exception of hepatic tumors.[18] The purpose of the current study was to describe conditions that may occur in a research colony of wild-caught ground squirrels, potentially resulting in death or the need for humane euthanasia. The species included in this study were primarily *U. richardsonii* and *U. columbianus*, with 6 *C. lateralis*.

Materials and Methods

The ground squirrel research colony reported here was maintained at the Department of Biologic Sciences, University of Alberta (U of A) from 1985 to 2001. Animals were maintained according to the guidelines of the Canadian Council on Animal Care (CCAC),[2] and research protocols were approved by the Biosciences Animal Care Committee (ACC) of the U of A. The numbers in the colony varied between 100 to 150 ground squirrels at any one time, with 200 being approved for use annually on ACC protocols. The U of A only maintains protocols, colony management, and individual animal records for 7 y, the retention period required by the CCAC. These records were no longer available for this retrospective study. However, we obtained postmortem records and histology slides from 1992 through 2001, the last decade of the colony's existence. We used these records to conduct the study.

Richardson ground squirrels were wild-caught on an ongoing basis from 2 local farms while Columbian and golden-mantled ground squirrels were trapped at a site in the foothills of the Rocky Mountains. The animals were captured using Havahart live catch traps (18″ × 5″ × 5″) made of wire mesh with a spring-loaded trap door and were transported to the U of A in rat cages in the back of a covered truck or van. The capture of all ground squirrels was conducted under permits from the Province of Alberta Fish and Wildlife Division.

Upon arrival at the animal facility, animals were housed singly in deep rat cages (8″ × 8″× 9″) with filter tops and either aspen chip or shavings as bedding. Water was provided via 250 mL mouse bottles (model 8-38S) with a bearing stopper sipper tube (model 5.5F) (Girton Manufacturing Company, Millville, PA).

Upon arrival, the technician played with the bottle stopper to show the squirrel where it was, and they had no problems finding the water source. Shredded copy paper and a plastic tube were added to the cages for enrichment. Animals were fed 5001 rodent diet (Canadian Lab Diet, 3030 50302 RR244A, Leduc County, Alberta, Canada T4× 0P4) with tap water supplemented with beet pulp pellets, fresh carrots, lettuce and sunflower seeds. Ground squirrels were weighed weekly and handled as much as possible. Eventually, most ground squirrels could be handled safely without gloves.

Ground squirrels were quarantined in isolation for 30 d. During this time, an animal health technologist conducted a physical examination, animals were ear-tagged, and antiparasite treatments were administered as necessary under the direction of a veterinarian. Most animals were infected with pinworms and coccidia upon arrival, and all were treated with a topical ivermectin spray 1:9 dilution (Ivomec, 1mg/mL, Merial 1010 72 Ave NE, Calgary, Alberta, Canada) both upon arrival and once a week to the end of the quarantine period. All were given a single oral dose of trimethoprim and sulfamethoxazole 5 mL/250 gm (Apo-Cotrimoxazole, Apotex150 Signet Drive, Toronto, Ontario Canada M9L 1T9). At the end of the quarantine period, all animals underwent fecal examination for endoparasites and skin scrapings for ectoparasites; a negative test was required before an animal was released to the main colony.

Upon relocation to the main colony, animals were housed in groups of up to 10 in 2 joined 90 × 90 × 90 cm meshed metal cages. Each cage had multiple levels with tubes, ramps, shelves, and wheels for enrichment. Occasionally, animals were given aspen tree branches on which to chew. Animals were fed as in the quarantine facility. Pans of autoclaved soil were placed as latrines in the corner of each cage complex for defecation and urination. These were used frequently, as ground squirrels are exceptionally clean animals. Incisor teeth of some squirrels required regular trimming. At capture, some squirrels had malocclusion and overgrown incisors that had to be trimmed. We surmised the overgrowth of incisors was due to a lack of bark and wood to wear down the animal's teeth. Therefore, we provided wood blocks to the animals in the later years of the colony. Problems with wood slivers from these blocks were encountered initially, and apple wood branches were found to be a better alternative than woodblocks. Teeth were not trimmed under anesthesia, as the latter appeared to be more stressful for the squirrel. The technicians became very adept at trimming teeth and performed the task quickly using sharp wire cutters. Squirrels that required trimming of incisors were given wet mash food option for 2 d after the procedure to alleviate any minor pain or discomfort.

Males and females of the same species were housed together, although ground squirrels were not intentionally bred in the colony. Some females were pregnant when they came into the colony or became pregnant; however, females rarely gave birth to live pups in the vivarium. When they did, pups were seldom found and were assumed to have been killed and eaten by adults. Only a single pup survived and grew to adulthood.

For research protocols involving hibernation, hibernation was induced by placing individual animals directly into the cold room at 4.9 to 5 °C in deep rat cages (8″ × 8″× 9″), with 1.5 in. thick aspen chip bedding mixed with aspen shavings on the bottom, some corn cob bedding in corners to absorb urine, and gray commercial sterilized recycled paper bedding with shredded paper on top. Their food was reduced to one pellet per day with full water and a small piece of carrot a few days before being placed in cold rooms. Once placed in cold rooms, water was removed and animals were left with one pellet of food for a 7 d acclimatization period. After this period, access to water was provided ad libitum, with 1 pellet in the feeder and 1 piece of carrot in the cage available for eating if desired. The carrot piece was changed whenever it dried out. During hibernation, animals were disturbed as little as possible.

A gross postmortem examination was performed on most animals that either died or were euthanized while in the colony between 1992 through 2001. A written report was completed for each animal, and these reports are the ones surveyed for this study. Information extracted from the reports included species, sex, time in the colony (if available), whether the animal died or was euthanized, type of research protocol to which each was assigned, and findings from the necropsy.

Microscopic slides of liver, kidney, heart, lung, spleen, brain, gastrointestinal tract, and other organs were prepared at the discretion of the pathologist on 100 animals. Tissues were fixed in 10% neutral buffered formalin for a minimum of 24 h, embedded in paraffin blocks, and processed routinely for microscopic examination. Five-micron-thick sections were stained with hematoxylin and eosin according to standard histology techniques. Additional histopathology and bacteriology studies were only performed occasionally. No virology procedures were performed.

Table 1. Postmortem findings in the colony of ground squirrels

Postmortem findings	No.[a]	Species[b]	Sex[c]
Kidneys, inflammation, glomerulo-interstitial, chronic; aorta, mineralization (2) and Kidney, medulla, inflammation, chronic active (1); Retroperitoneal abscess, eroded artery with rupture, acute (1); cellulitis (1); abdomen, mass (1); aorta, media, mineralization, severe (2)	13	Rx 4 Cx7 GM U	Fx8 Mx4 U
Liver, adenoma(s); Skin, inflammation, subacute, locally extensice, severe (3); hemoperitoneum, rupture (1); peritonitis, suppurative, *Staphylococcus aureus* (1); kidney, atrophy, bilateral, chronic (1); Ear, inner, inflammation, bilateral, chronic, severe (1)	11	Rx5 Cx4 GMx2	Fx5 Mx3 Ux3
Liver, carcinoma, hepatocellular, metastatic; Kidney, inflammation, glomerulo-interstitial, chronic (1)	2	C GM	Fx2
Lymphoma, disseminated, severe; Spleen, rupture, lymphoma (2)	3	Rx2/C	Fx2 U
Liver, regeneration, nodular, generalized	1	R	F
Pleura, inflammation, fibrinous, acute	1	R	M
Intestine, volvulus, acute; abdomen, abscess	1	C	F
Pyothorax	1	R	M
Tongue, necrosis, chronic, severe; Colon, inflammation, acute, mucoid	1	R	F
Mesometrium, abscess, multiple; *Arcanobacter pyogenes*	1	C	F
Lung, bronchi, inflammation, suppurative, acute	1	C	M
Hemothorax, cause undetermined	1	R	F
Heart, inflammation, subacute, severe	1	R	M
Skin, ventral abdomen, inflammation, severe	1	C	M
Skin, inflammation, extremities, moderate; nail beds, inflammation, severe (1)	2	Cx2	Fx2
Colon, inflammation, catarrhal, acute	1	R	F
Skin, inflammation, periorbital subacute, locally extensive, severe	1	C	M
Abscesses, multiple organs; Kidney, medulla, inflammation, suppurative, severe	1	R	F
Lung, thrombosis, septic, acute	1	R	U
Kidney, infarction, unilateral, acute; Perirenal tissue, hemorrhage;	1	R	F
Lung, pneumonia, interstitial, subacute; Subcutaneous abscess and pyelitis (1); Liver, inflammation, suppurative, multifocal (1)	2	R C	F M
Uterus, inflammation, suppurative, acute	1	R	F
Lung, abscess, rupture	1	R	U
Appendicular skeleton, joints, inflammation, chronic, moderate	1	R	F
Kidneys, infarcts, chronic, bilateral; abscess, peri-renal (1)	2	C /GM	F/M
Oral cavity, inflammation, necrotizing, acute, severe, with toxemia	1	R	F
Tongue, inflammation, diffuse, severe, *Candida albicans*	1	C	F
Heart, left ventricle, hypertrophy, chronic; aorta, media, mineralization, diffuse, severe (2); diabetes mellitus (2)	3	R Cx2	Mx3
Hypothermia, metabolic chamber malfunction	1	C	M
No diagnosis; fighting prior to death (1)	4	Rx3/U	F Mx2 U
Meninges, inflammation, subacute, locally extensive, severe; teeth, incisors, malocclusion	1	R	M
Uterus, leiomyoma	1	GM	F
Spleen, inflammation, necrotizing, acute, severe; connective tissues, inflammation, suppurative, generalized, severe	1	R	F
Heart, right ventricle, dilation, moderate; Right AV valve, anomaly, small cusps, moderate (1)	2	Rx2	F M
Pericardium, inflammation, acute, fibrinous, severe; ear, external, inflammation, unilateral, acute, severe (1)	2	R C	Fx2
Totals	70	R = 35 C = 27 GM = 6 U = 2	F = 39 M = 23 U = 8

[a]Animals with the same condition are grouped as much as possible. The number given is the total with this condition. When such animals also have other conditions, these are listed on the appropriate line with the number affected following each diagnosis in brackets.
[b]U = not recorded, R = Richardson's, C = Columbian, GM = golden-mantled
[c]U = not recorded, M = male, F = female

Results

A total of 160 ground squirrels was submitted for postmortem examination. Of these, 119 were Richardson's, 31 were Columbian, 6 were golden-mantled, and for 4 animals, the species was not recorded. There were 62 males, 85 females, and 13 for which the sex was not recorded. Age of animals was not determined, but all were fully grown and considered to be adults. A total of 126 animals were used in metabolism studies, 22 in liver transplant studies and 2 in vaccine development, while 10 individuals had not been assigned to a research protocol at the time of death.

Of the 160 animals submitted for postmortem examination, 51 were found dead and 109 were euthanized. Toward the end of the metabolism research program, 58 animals in the colony were culled to reduce colony size. These animals were all clinically normal at the time of euthanasia. This group of 58 was included in the 109 euthanized animals in this study. An exact mortality rate could not be determined because only a few experiments were terminal, and many animals had already lived several years in the colony. However, a rough estimate is that these 160 animals, less the 58 clinically normal culls, represent less than 10% of the population of the colony during the 10y span of the study.

The gross postmortem findings in all animals are grouped into 4 categories: the general population of the colony (Table 1); those that died or were euthanized during the quarantine period (Table 2); those that were normal animals that were culled when colony numbers were reduced (Table 3) and those associated with a liver transplant protocol (Table 4). For each animal, sex, species, and time in the colony, when known, were correlated with the pathologic findings. We saw no apparent association between pathologic findings, sex, and species.

The 2 most common pathologic findings were chronic glomerulo-interstitial nephritis and hepatocellular tumors. Chronic glomerulo-interstitial nephritis was characterized grossly by bilaterally small, pale, fibrous kidneys, with numerous spherical cortical cysts generally of less than 2 mm diameter, but occasionally as large as 3 mm diameter. When sections from animals with such gross findings were available for microscopic examination, glomerular and interstitial lesions were found to coexist. A progression of kidney lesions was evident, with an early stage characterized by prominent glomeruli, mesangial thickening and cortical interstitial infiltrates of lymphocytes. The lesion progressed through a stage of ongoing mesangial thickening with distention of the glomerular space, adhesion of the glomerular tuft to Bowman's capsule, thickening of Bowman's capsule and accumulation of peri-glomerular lymphocytes. The cortex had interstitial lymphocyte infiltrates, early dilation of tubules and tubular cast formation. Further progression of the lesion resulted in additional mesangial thickening, a greater degree of adhesion of the glomerular tuft to Bowman's capsule, epithelialization of Bowman capsule and more extensive periglomerular lymphocyte infiltration. Cortical damage progressed to continued interstitial lymphocyte infiltration with more tubular casts, atrophy of cortical tubules, foci of interstitial mineral deposition and large cortical tubular cysts. In the final stage of the process, all of the previous changes were present, along with extensive cortical atrophy and glomerular sclerosis (Figure 1).

The second most common finding was that of hepatic masses. For the purposes of this study, hepatocellular masses were classified as adenomas if they showed nodular parenchymal swelling, adenocarcinomas if metastasis outside the liver occurred, and nodular regeneration if hepatocyte proliferation and severe fibrosis were both present in the liver. Hepatocellular masses were common and were present as both single and multiple lesions. They measured as much as 3 cm diameter and were most commonly distinct with clear demarcation of the edges from the surrounding normal hepatic parenchyma. Occasionally the boundaries were indistinct, especially if lesions were multiple and abutting one another. In a few instances, boundaries were poorly delineated because they appeared to be locally invasive. However, only 2 animals had tumors that had metastasized elsewhere in the body. Histopathology of liver masses revealed the full spectrum of changes described in detail in ground squirrels by other authors (Figure 2 A).[18] The morphology of the metastatic lesions in the 2 affected ground squirrels was unexpectedly benign (Figure 2 B).

Table 2. Postmortem findings: ground squirrels in quarantine (first month of captivity)

Postmortem findings	No.[a]	Species[b]	Sex[c]
Colon, inflammation, acute mucoid, etiology undetermined	4	Rx3	Mx2
		U	F
			U
Trauma	2	R	U
		C	M
Aorta, stenosis, moderate	1	U	F
Emaciation/ pregnant	1	R	F
Heart, failure, acute, cause undetermined	1	U	U
Totals	9	R = 5	F = 3
		C = 1	M = 3
		U = 3	U = 3

[a]Animals with the same condition are grouped as much as possible. The number given is the total with this condition. When such animals also have other conditions, these are listed on the appropriate line with the number affected following each diagnosis in brackets.
[b]U = not recorded, R = Richardson's, C = Columbian, GM = golden-mantled
[c]U = not recorded, M = male, F = female

A wide variety of other pathologic conditions was also found, but none were as common as the kidney and liver lesions. The 58 clinically normal animals that were culled (Table 3) provide a snapshot of the overall pathology that might occur in a clinically normal population of wild caught animals. Of the culled animals, 43 (74%) had no gross postmortem abnormalities. This suggests that approximately 75% of the animals in the colony would be expected to be normal. Of the remainder, 4 had with chronic glomerulo-interstitial nephritis, 3 of which also had hepatic adenomas, another 3 had hepatic adenomas, and 2 had mottled/swollen livers that might have been adenomas, but were not confirmed by histology. Three ground squirrels had dermatitis, 1 had sperm granulomas in the vas deferens, another had an unidentified omental mass, and a third had pulmonary microlithiasis. The 22 ground squirrels that were used in liver transplant studies mostly died or were euthanized due to transplant rejection or postoperative complications such as renal tubular necrosis and fibrinous perihepatitis (Table 4).

Time in the colony was not included in the postmortem report for 94 animals, but was available for 66. Of these, 9 died or were euthanized during the 1 mo quarantine period (Table 2) and 57 died/were euthanized at some point after completing quarantine: 9 within 1 to 6 mo, 29 between 7 and 24 mo, 8 between 25 and 48 mo, and 11 after more than 48 mo in the colony. The longest surviving Richardson's ground squirrel was in the colony for 30 mo, while 3 Columbian and 5 of 6 golden-mantled squirrels survived beyond 48 mo. For those animals with a known time of residence in the colony, the most common

Table 3. Postmortem findings: ground squirrels that were clinically normal when culled from colony

Postmortem findings	No.[a]	Species[b]	Sex[c]
Normal/No gross postmortem findings	43	Rx42	Fx32
		U	Mx8
			Ux3
Nephritis, glomerulo-interstitial, chronic; Liver, adenoma(s), multiple (3); skin, subcutaneous, abscess (1)	4	Rx4	Fx3
			M
Liver, adenoma(s); infarcts, kidneys, bilateral, chronic, moderate(1)	3	GM, Rx2	U, F, M
Skin, ventral midline, alopecia, severe	3	Rx3	Fx3
Liver, left lateral lobe, swollen, mottled	2	Rx2	M F
Omentum, mass, undetermined cause	1	R	M
Vas deferens, granuloma, sperm, chronic	1	R	M
Lung, alveoli, microlithiasis, multifocal, mild	1	R	M
Totals	58	R = 56	F = 40
		GM = 1	M = 14
		U = 1	U = 4

[a]Animals with the same condition are grouped as much as possible. The number given is the total with this condition. When such animals also have other conditions, these are listed on the appropriate line with the number affected following each diagnosis in brackets.
[b]U = not recorded, R = Richardson's, GM = golden-mantled
[c]U = not recorded, M = male, F = female

Table 4. Postmortem findings: liver transplant ground squirrels (most died/were euthanized due to transplant rejection or postoperative complications)

Postmortem findings	No.[a]	Species[b]	Sex[c]
Peritonitis/perihepatitis, suppurative, acute, severe; Kidney, tubules, necrosis, acute(2)	5	Rx5	Mx5
Kidney, inflammation, glomerulo-interstitial, chronic; anomaly, right atrium (1)	2	Rx2	Fx2
Transplant rejection, acute	2	Rx2	M/F
Liver, inflammation, suppurative, diffuse, acute	2	Rx2	Mx2
Liver, necrosis, acute, severe	1	R	M
Shock, postoperative	1	R	M
No diagnosis	1	R	M
Colon, inflammation, mucoid, acute	1	R	M
Dehydration	1	R	M
Kidney, tubules, cortical, necrosis, acute;	2	Rx2	Mx2
Pericarditis, fibrinous, acute, severe	1	R	M
Heart, inflammation, lymphocytic, multifocal, minimal; lung, inflammation, bronchi, suppurative, locally extensive, mild	1	R	M
necrosis, tongue, chronic, severe	1	R	M
Spleen, hemangiosarcoma, metastatic	1	R	M
Totals	22	R = 22	F = 3
			M = 19

[a]Animals with the same condition are grouped as much as possible. The number given is the total with this condition. When such animals also have other conditions, these are listed on the appropriate line with the number affected following each diagnosis in brackets.
[b]R = Richardson's
[c]M = male, F = female

postmortem findings differed with duration of captivity. During the first month in the colony (quarantine, Table 2), mucoid colitis was the most common disease (4 of 9 animals) and trauma was the second most common (2 of 9). In the time between 2 and 6 mo and 7 to 24 mo in the colony, a wide variety of conditions was observed (Table 5). However, between 7 and 24 mo, the 2 most common conditions emerged: chronic glomerulo-interstitial nephritis (6 of 30) and hepatic adenomas (4 of 30). In the 25 to 48 mo group, chronic glomerulo-interstitial nephritis was by far the most common finding, being present in 7 of 8 ground squirrels. Only 1 animal in this group had an hepatic adenoma. After 48 mo in the colony, the frequency reversed, with 6 of 11 having hepatic tumors compared with 2 of 11 with chronic glomerulo-interstitial nephritis (Table 5). The numbers and percentages of animals with hepatic masses and glomerulo-interstitial nephritis when time in the colony was known are presented in Table 6.

Discussion

The purpose of this retrospective study was to document the pathologic conditions that occur in captive ground squirrels. As ground squirrels seldom produce fertile matings in captivity, colonies must be maintained by capture from the wild. This prevents forming a closed colony of known disease status, which is standard in other rodent research colonies. In the wild, ground squirrels are subject to plague, tularemia, encephalomyocarditis,

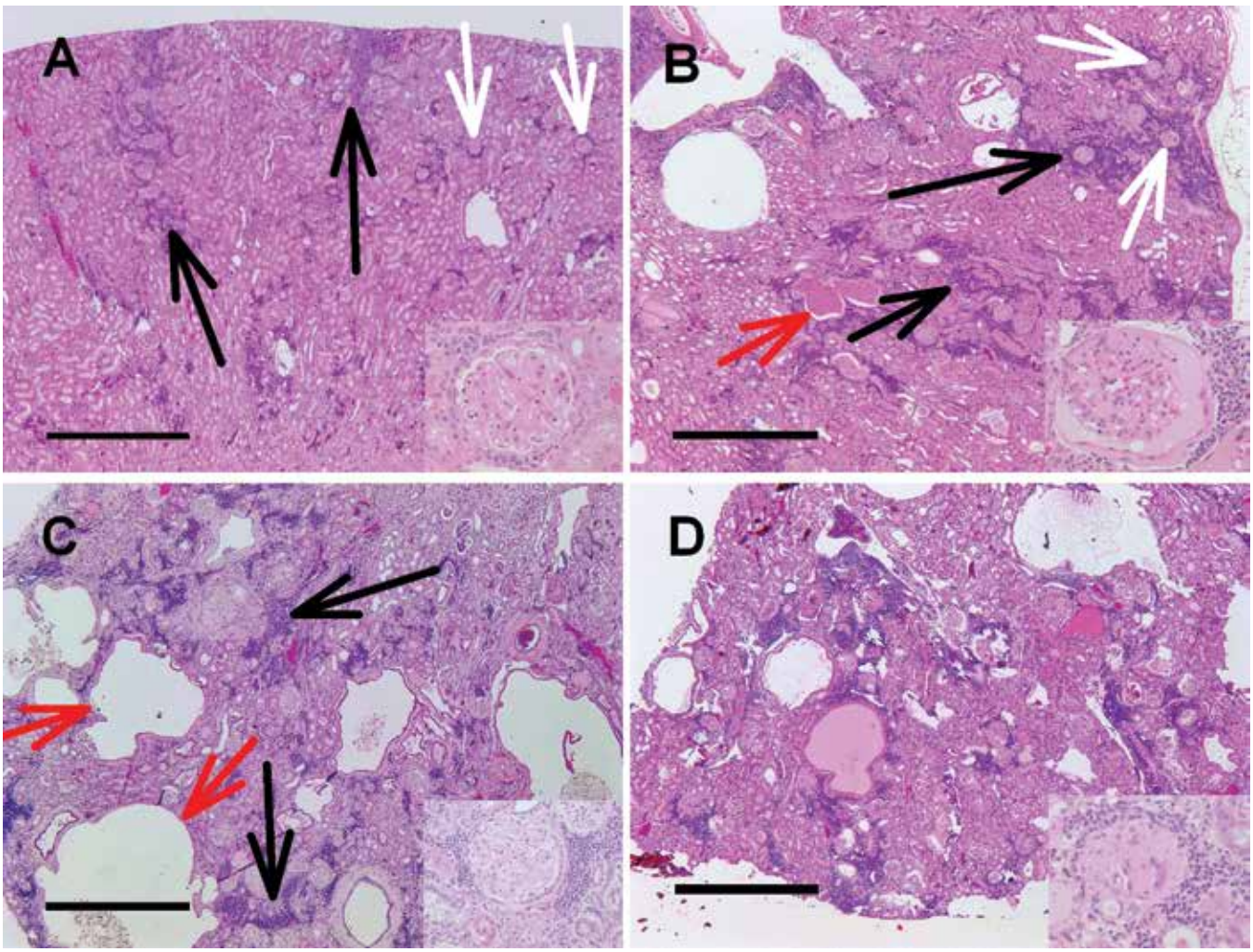

Figure 1. Progression of kidney lesions in development of glomerulo-interstitial nephritis in ground squirrels. (A) Early stage of glomerulo-interstitial nephritis with prominent glomeruli having mesangial thickening (white arrows) and interstitial lymphocyte infiltrates (black arrows). Inset: glomerulus with mesangial thickening. Columbian ground squirrel, female, unknown time in colony. (B) Progression of renal lesions. Prominent glomeruli (white arrows), interstitial lymphocytes (black arrows), tubular cast formation (red arrows). Inset: glomerulus with mesangial thickening, distention of the glomerular space, adhesion of glomerular tuft to Bowman's capsule, thickening of Bowman capsule and periglomerular lymphocytes. Columbian ground squirrel, female, 25 mo in colony. (C) Further progression of renal lesions. Interstitial lymphocytes (black arrows), atrophy of cortical tubules and large cortical tubular cysts (red arrows). Inset: Glomerulus with further mesangial thickening, a greater degree of adhesion of glomerular tuft to Bowman capsule, epithelialization of Bowman capsule and more extensive periglomerular lymphocyte infiltration. Columbian ground squirrel, female, 30 mo in colony. (D) End stage of process. All the changes in C with extensive cortical atrophy. Inset: sclerotic glomerulus with Bowman's capsule overrun by lymphocytes. Columbian ground squirrel, female, 48 mo in colony. All photomicrographs 20×, Bar = 1 mm. Insets, 400×

Ground Squirrel Hepatitis Virus (GSHV),[18] Rocky Mountain spotted fever and *Streptobacillus moniliformis* infections among others. Parasites of *U. richardsonii* have been described in the wild in Alberta[13] and cover a wider range than represented in this study. Periodic introduction of infectious disease and parasites is thus an ongoing risk to an experimental colony. Colony management procedures are designed to reduce the chance of direct spread of infectious disease and to eliminate intermediate hosts required by parasites. For the colony that was the subject of this study, a 30-d isolation and quarantine period was adequate, and none of the above diseases or agents was found in the captive population described herein. However, microbiology was seldom performed on this colony, and no animals were tested for Ground Squirrel Hepatitis Virus (GSHV). In addition, a routine fecal examination was not performed on animals in the main colony, as all animals were routinely treated for parasites upon arrival and confirmed negative by fecal flotation at the end of the quarantine period.

We assume that any pathology of ground squirrels discovered in the first month in the colony (quarantine period) would indicate diseases that developed in the wild. Such information is important because subclinical disease in wild-caught animals is a potential confounding factor in biomedical research. In the first month, mucoid colitis was the most common finding (4 animals) and trauma (2 animals) was the second most common condition. Mucoid colitis is speculated to be due to stress, perhaps related to the stress of capture, as it occurred in only one other animal after the quarantine period. Of the 2 injured animals, 1 most likely was injured at the time of capture, while the other became caught in the lid of its cage later in the quarantine period. Both were euthanized for humane reasons. The remaining 3 animals do not form a large enough group to indicate what diseases might be common in the wild population.

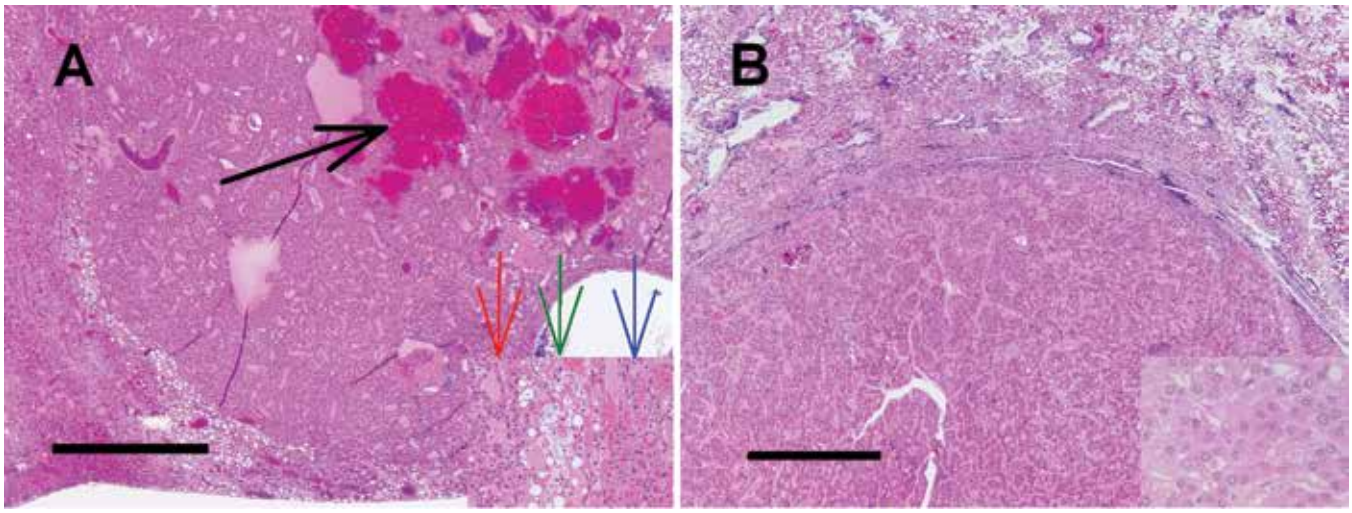

Figure 2. (A) Hepatocellular mass in the liver. Note that in addition to the hepatocellular mass there are variably sized blood-filled lacunae (black arrow). 20×, Bar = 1 mm. Inset: zone between the mass and normal hepatic parenchyma. Tumor (red arrow), cells containing lipid between tumor and normal parenchyma (green arrow) and normal parenchyma (blue arrow). 200×. (B) Hepatic adenocarcinoma metastatic to the lung. 20×, Bar = 1 mm. Note the relatively benign histologic appearance of the metastatic mass (inset 400×).

Therefore, insufficient numbers of animals were available in the 1 mo quarantine period to provide an accurate assessment of disease in the wild ground squirrel population and the potential threat endemic disease would pose to biosecurity and research protocols. One animal that had aortic stenosis survived in the wild until captured.

Few other reports document disease in captive ground squirrels. Most of these are case reports of individual or small numbers of animals.[16] These have reported spontaneous obesity, formation of gallstones[17] and hepatocellular carcinomas.[15] In the current study, apart from the quarantine period, we observed a wide variety of pathologic conditions. We did not find any correlation between pathologic findings and either species or sex. However, 2 pathologic conditions were more common than the rest. The urinary tract was the most frequently affected organ system, with chronic glomerulo-interstitial nephritis being the most common disease process overall. Affected animals appeared to show a clear progression of this disease, with glomerular and interstitial lesions progressing in concert toward an end-stage of cortical atrophy, cysts, and fibrosis. No etiology was evident for this process. Of those animals for which the time in the colony was known, the percentage of animals having this condition increased with time in the colony, decreasing only after 48 mo (Table 5). It is possible that chronic glomerulo-interstitial nephritis decreased as a percentage of losses after 48 mo because morbidity and mortality due to this condition resulted in removal of most affected animals prior to 48 mo.

Liver tumors were the second most common pathologic finding in our ground squirrel colony. Such tumors are common in ground squirrels and GSHV is a potential etiologic agent.[10] These tumors are often classified as hepatocellular carcinomas (HCC), and both HCCs and adenomas have been described in great detail in the Richardson's ground squirrel.[18] However, the criteria for classifying HCC and distinguishing it from hepatic adenoma (HA) were not provided in either of these studies.[10,18] Furthermore, these studies did not report any extrahepatic metastases. In the present study, several animals had hepatocellular masses. Although many were 2 to 3 cm diameter, they appeared to be clinically silent, indicating that their volume was insufficient to disrupt normal hepatic function. The hepatic masses of animals in this study had all of the various microscopic changes described previously.[18] Only 2 animals had extra-hepatic metastases, and as defined for our study, only these 2 would qualify as HCC, as metastasis is considered the unequivocal criterion of malignancy. A striking feature of the 2 HCC in this study was the relatively benign, adenoma-like appearance of the metastases (Figure 2 B). This observation calls into question the sole use of microscopic criteria to distinguish HCC from HA. Of those animals whose time in the colony was known, hepatic tumors were not seen until the 7 to 24 mo period, when 4 of 30 animals (13%) had either an hepatocellular adenoma or carcinoma as either the primary or concurrent finding. Between 25 and 48 mo only 1 animal had a hepatoma (12.5%) but after 48 mo, 6 animals had hepatomas (54.5%). This increase in percentage after 48 mo might be the result of losses due to chronic interstitial nephritis removing affected animals at an earlier date, while animals with adenomas, a more benign condition, survived for a longer period. Animals may also have a greater chance of neoplasia developing with advancing age, and these 2 processes may both occur. The lack of tissues stained for GSHV is a weakness of this retrospective study; unfortunately, fixed or paraffin embedded tissues were not available for retrospective testing for GSHV.

This study deals with ground squirrels in a research colony. As research animals they receive a high standard of care and nutrition and freedom from predation. Consequently, the diseases that they develop in the colony do not necessarily reflect those that would occur in wild populations. Therefore, the present findings cannot be extrapolated to wild ground squirrels. Likewise, the animals in this study that died during quarantine from conditions that were not stress related or traumatic were too few to give any indication of what the disease situation might be in the wild. The documents used for this study are "after the fact" and therefore, have deficiencies in the information available. Specifically, knowing whether animals were infected with GSHV would be useful. These areas of investigation remain open to others who have similar colonies of ground squirrels that can be followed prospectively.

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Table 5. Postmortem findings in ground squirrels that died/were euthanized when length of residence in colony was available

Time[a]	Postmortem findings[b]	No.	Species[c]	Sex[d]
2 – 6 mo	Kidneys, inflammation, glomerulo-interstitial, chronic; Retroperitoneum, abscess, hematoma, eroded artery with rupture	1	R	F
	Lung, thrombus, septic, acute	1	R	U
	Kidney, infarct, acute, perirenal hemorrhage[1]	1	R	F
	Kidneys, infarcts, chronic, bilateral, severe	1	C	M
	Kidneys, tubules, necrosis, acute, post transplant	1	R	M
	Lung, interstitium, inflammation; Heart, left ventricle, hypertrophy, moderate	1	C	M
	Uterus, inflammation, suppurative	1	R	F
	Lung abscess, ruptured	1	R	U
	Joints, inflammation, chronic, multiple	1	R	M
	Totals	9	R = 7	F = 3
			C = 2	M = 4
				U = 2
7 – 24 mo	Kidneys, inflammation, glomerulo-interstital, chronic; skin, inflammation, ulcerative, moderate (1); Digits, inflammation, multifocal, moderate, chronic (1); Liver, adenoma (1)	6	Cx2	Fx3
			Rx4	Mx2
				U
	Liver, adenoma/s; adenocarcinoma (1); ruptured (1);	3	Rx3	Fx3
	No diagnosis; wounds, fighting (1)	3	Rx3	MX2/F
	Heart, pericardium, acute, fibrinous, acute	2	Rx2	M/F
	Skin, inflammation, moderate; interface	2	C/R	F/U
	Kidneys, hypoplasia, bilateral;, moderate	1	R	F
	Oral cavity, inflammation, chronic, severe; toxemia	1	R	F
	Meninges, inflammation, suppurative, acute, severe	1	R	M
	Uterus, leiomyoma	1	C	F
	Connective tissues, inflammation, suppurative, severe	1	R	F
	Oral cavity, mucosa, inflammation, *Candida albicans*	1	C	F
	Lungs, inflammation, interstitial, chronic, moderate	1	C	M
	Pleura, inflammation, fibrinous, acute	1	R	M
	Lymph nodes, inflammation,	1	R	U
	Heart, hypertrophy, left ventricle, moderate	1	R	M
	Heart, right ventricle, failure, chronic, moderate	1	R	M
	Heart, atrioventricular valves, endocardiosis, moderate	1	R	F
	Lymphoma, generalized	1	R	F
	Hemangiosarcoma, metastic	1	R	M
	TOTALS	30	R = 24	F = 16
			C = 6	M = 11
				U = 3
25 – 48 mo	Kidneys, inflammation, glomerulo-interstitial, chronic; peritoneal cavity, mass, undetermined (1); connective tissues, inflammation, diffuse, moderate (2); aorta, media, mineralization (1);	7	Cx7	Ux2
				Fx4
				M
	Liver, adenomas; peritoneum, inflammation, suppurative, scute, *Staph aureus*	1	C	M
	Totals	8	C = 8	F = 4
				M =2
				U =2
48 mo	Liver, adenoma/s; kidneys, atrophy, chronic, prior infarction (1); ear, middle, inflammation, chronic, moderate (1)	5	Cx2	Mx2
			GMx3	F
				Ux2
	Kidneys, inflammation, glomerulo-interstitial, chronic; aorta, media, mineralization	2	GM/U	F/U
	Liver, adenocarcinoma. metastatic	1	GM	F
	Peri-renal tissue, abscess; kidney, infarcts, chronic	1	GM	F
	Heart, left ventricle, hypertrophy, moderate; aorta, media, mineralization, diffuse	1	C	F
	Lymphoma, generalized	1	GM	F

Table 5. Continued

Time[a]	Postmortem findings[b]	No.	Species[c]	Sex[d]
Totals		11	C = 3	F = 6
			GM = 7	M = 2
			U = 1	U = 3

[a]Time in colony
[b]Animals with the same condition are grouped as much as possible. The number given is the total with this condition. When such animals also have other conditions, these are listed on the appropriate line with the number affected following each diagnosis in brackets.
[c]U = not recorded, C = Columbian, GM = golden-mantled, R = Richardson's
[d]U = not recorded, M = male, F = female

Table 6. Ground squirrels with hepatic tumors and glomerulo-interstitial nephritis compared with time in colony

Time in colony (mo)	No. of animals in cohort	Adenoma/carcinoma no. (%)	Glomerulo-interstiital nephritis no. (%)
< 1	9	0 (0%)	0 (0%)
2 – 6	9	0 (0%)	1 (11%)
7 – 24	30	4 (13%)	6 (20%)
25 – 48	8	1 (12.5%)	7 (87.5%)
> 48	11	6 (54.5%)	2 (18%)

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