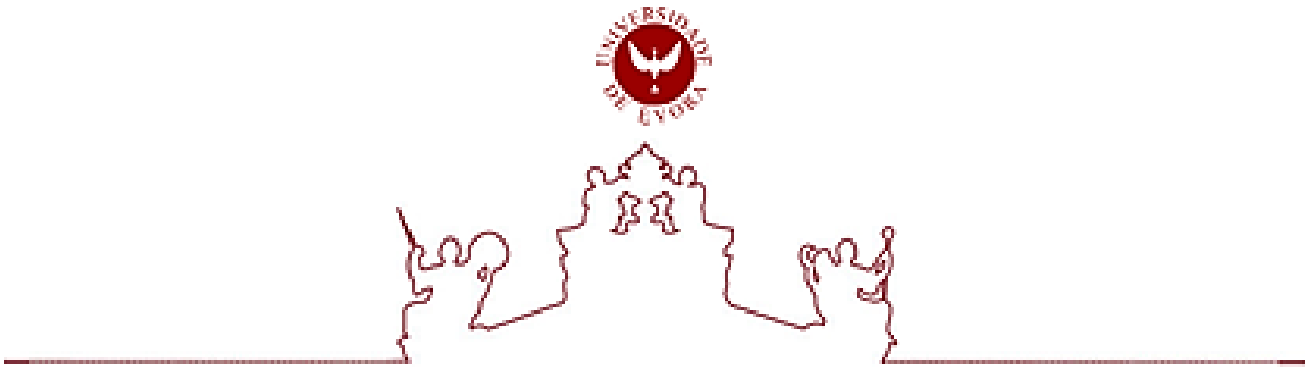

Universidade de Évora - Escola de Ciências e Tecnologia

Mestrado Integrado em Medicina Veterinária

Relatório de Estágio

Feline Chronic Kidney Disease

Luís Tomás Torres

Orientador(es) | Luís Miguel Lourenço Martins

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Évora 2024

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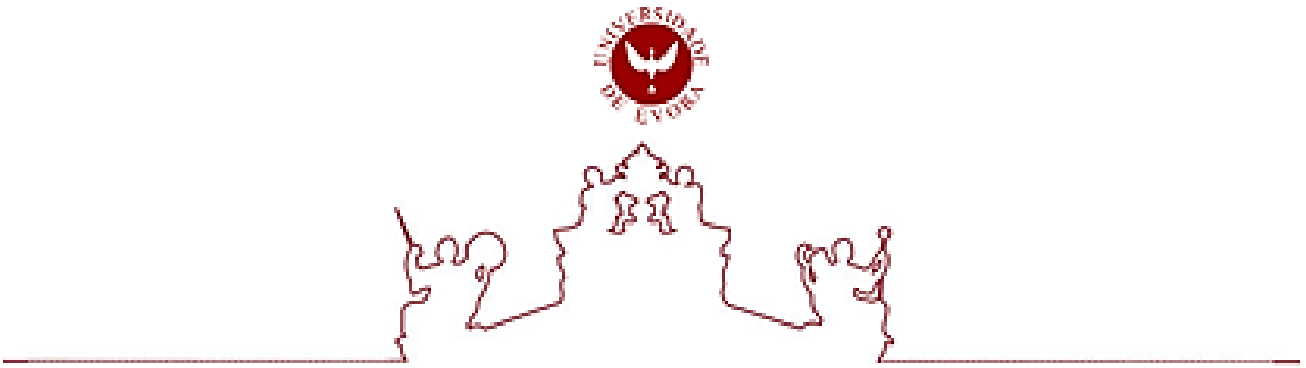

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Évora 2024

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Finally, to my parents and brother, for all of the love and support that they bestowed on me, for making me the person I am and for their education and presence, teaching me that I need to work hard to achieve my goals and dreams.

Abstract

The present report emerges as a result of the six-month period traineeship held at Donaldson's Vets, in the United Kingdom, in regard to the Master's degree in Veterinary Medicine from the University of Évora. The report is divided into three parts. The first is a descriptive analysis of the clinical cases observed during the probationary period by the trainee. The second is a monograph on the matter of Feline Chronic Kidney Disease, addressing the renal anatomy and physiology, pathophysiology, potential comorbidities and complications of the disease, diagnostic and treatment approaches, as well as its monitoring. Finally, the third part is the presentation and discussion of two clinical cases accompanied by the author. The first about a recently diagnosed case and the second about a stable life-long patient.

Keywords: feline, chronic, kidney, senior, monitoring

Resumo – Doença Renal Crónica Felina

O presente relatório surge em resultado do estágio curricular de seis meses realizado no hospital Donaldson's Vets, no Reino Unido, no âmbito do Mestrado Integrado em Medicina Veterinária da Universidade de Évora. O relatório encontra-se dividido em três partes. Na primeira é realizada uma análise descritiva dos casos clínicos observados durante o período de estágio. A segunda é uma monografia sobre o tema Doença Renal Crónica Felina, onde, em forma de revisão bibliográfica, são abordadas a anatomia e fisiologia renal, a patofisiologia, potenciais comorbilidades e complicações da doença, abordagens para o diagnóstico e tratamento e a monitorização da doença. Por fim, é feita a apresentação de dois casos clínicos seguidos pelo estagiário durante o estágio. O primeiro de um gato recentemente diagnosticado e o segundo sobre um paciente de longa-data com doença estável.

Palavras-chave: felina, crónica, renal, senior, monitorização

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List of Abbreviations

ACE: angiotensin-converting enzyme

ACEI: angiotensin converting enzyme inhibitor

ADH: antidiuretic hormone

AKI: acute kidney injury

ANP: atrial natriuretic peptide

ARB: angiotensin receptor blockers

AT1: angiotensin II type 1 receptors

ATP: adenosine triphosphate

BUN: blood urea nitrogen

Ca^{2+}: calcium ion

CKD: chronic kidney disease

Cl^-: chloride ion

Cl: chloride

CO: carbon monoxide

CO_2: carbon dioxide

COX-2: cyclooxygenase-2

CRFK: Crandal-Rees feline kidney

ECM: extracellular matrix

ELISA: enzyme-linked immunosorbent assay

EMT: epithelial-mesenchymal transition

EPO: renal erythropoietin

ESL: endothelial surface layer

FeLV: feline leukaemia virus

FGESF: feline gastrointestinal eosinophilic sclerosing fibroplasia

FGF-23: fibroblast growth factor 23

FiV: immunodeficiency virus

GBM: glomerular basement membrane

GFR: glomerular filtration rate

GSH:GSSG: reduced/oxidized glutathione ratio

H^+: hydrogen ion

H_2O: water

$H_2PO_4^-$: phosphoric acid

HCO_3^-: bicarbonate ion

HPO_4^{2-}: hydrogen phosphate

IRIS: International Renal Interest Society

K: potassium

K^+: potassium ion

KCl: potassium chloride

Mg^{2+}: magnesium ion

MSC: mesenchymal stem cell

Na: sodium

Na^+: sodium ion

Na-K-ATPase: sodium-potassium-adenosine triphosphatase

NaCl: sodium chloride

NH_3: ammonia

NH_4^+: ammonium

NKCC2: Na-K-2Cl cotransporter

NO: nitric oxide

OH^-: hydroxyl ion

PTH: parathyroid hormone

PBA: phosphate binding agents

PCV: packed cell volume

PGE_2: prostaglandin E_2

Pi: inorganic phosphate

PTH: parathyroid hormone

PU/PD: polyuria/polydipsia

RAAS: renin-angiotensin-aldosterone system

ROS: reactive oxygen species

RSPCA: Royal Society for the Prevention of Cruelty to Animals

SDMA: symmetric dimethylarginine

TGF-β1: transforming growth factor – β1

TPLO: tibial plateau levelling osteotomy

TSAT: transferrin saturation

UPC: protein/creatinine ratio

USG: urine specific gravity

UTI: urinary tract infections

VGG: Vaccination Guidelines Group

WSAVA: World Small Animal Veterinary Association

I. Introduction

The present report was developed in order to conclude the Master's degree in Veterinary Medicine from the University of Évora, subsequently to the traineeship at Donaldson's Vets, in the United Kingdom, about clinical and surgical medicine in small animals, under the internal guidance of professor Luís Martins and the external supervision of Dr. Martin Paterson.

The traineeship took place from the 1st of October 2022 until the 31st of March 2023, making a total of six months and a workload of approximately 780 hours. Throughout the traineeship, the author was able to accompany the clinicians, spending most of the mornings assisting with the surgical procedures and dedicating the afternoons to observing and collaborating with internal medicine consults, being the latter the area of most interest to the author.

Throughout the stipulated period, the student was constantly involved in both surgical and internal medicine areas, encouraging him to deepen both theoretical and practical knowledge and develop clinical reasoning, which are important cornerstones necessary for his professional future.

The report is divided into three parts. The first is a descriptive analysis of the clinical cases observed during the probationary period, as well as a summarized description of the most relevant diseases and procedures accompanied by the trainee. The second is a monograph about the theme Feline Chronic Kidney Disease, where, as a literature review, the author addresses the anatomy and physiology of the kidneys, pathophysiology, potential comorbidities and complications of the disease, diagnostic and treatment approaches, and follow-up. Finally, the third part is the presentation and discussion of two clinical cases accompanied by the trainee. The first about a stable life-long patient and the second about a recently diagnosed case.

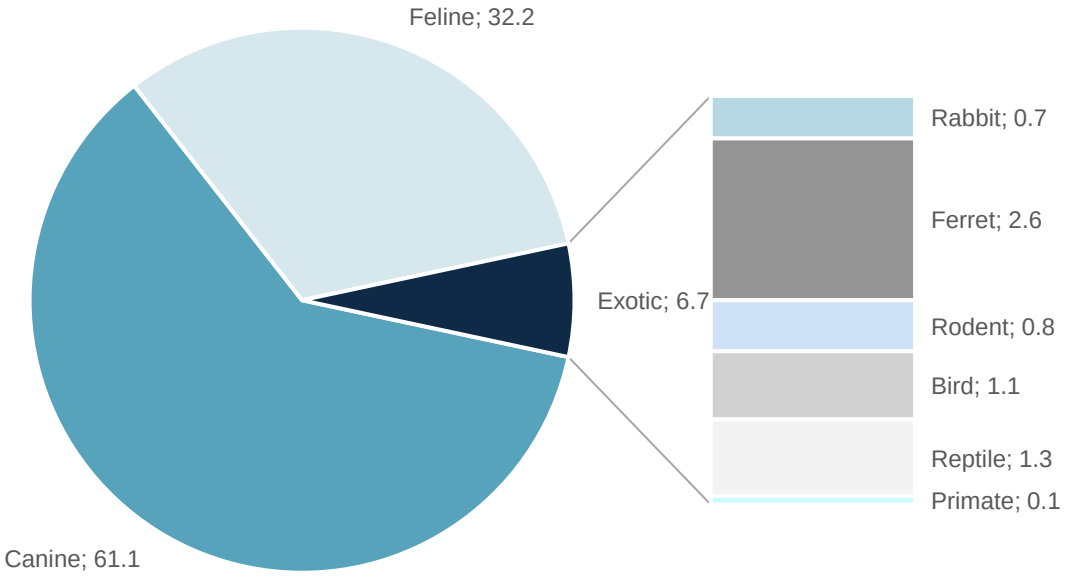
II. Traineeship report

The following traineeship report portrays a descriptive analysis of the cases accompanied by the author during the six-month period at Donaldson's Vets. The data is divided into five categories: preventive medicine, clinical medicine, surgical medicine, imaging procedures, and medical procedures, which are further subdivided into more specific categories, depending on the identified disease. This information is arranged into graphics and tables containing data regarding the absolute frequency by species (Fip), the absolute frequency (Fi), as the sum of the Fip, and the relative frequency [Fr(%)], which represents the percentage of Fi in the totality of the cases.

It is noteworthy that the total number of the reported cases may not reflect the true inflow of patients to the practice, as not all patients that visited the practice were recorded. Furthermore, it is also important to consider that multiple consults and procedures happen at the same time throughout the working day, hence being impossible to obtain accurate information about the daily case load.

1. Case distribution

Through the statistical analysis of the animals observed during the traineeship, 717 in total, within the six-month period, it is possible to affirm that the majority of cases attended belonged to dogs (*Canis lupus familiaris*) and cats (*Felis catis*), which represent 61.1% and 32.2% of the patients observed, respectively. The remaining 6.7% include: five rabbits (*Oryctolagus cuniculus domesticus*), 19 ferrets (*Mustela putorius furo*), three guinea pigs (*Cavis porcellus*), one domestic rat (*Rattus norvegicus*), one hedgehog (*Erinaceus europaeus*), one chinchilla (*Chinchilla lanigera*), three chickens (*Gallus gallus domesticus*), one pidgeon (*Columba oenas*), one owl (*Tyto alba*), two parrots (*Ara chloropterus* and *Amazona aestiva*), one parakeet (*Melopsittacus undulatus*), four bearded dragons (*Pogona vitticeps*), one leopard geko (*Eublepharis macularius*), one corn snake (*Pantherophis guttatus*), one butterfly agama (*Leiolepis belliana*), and one royal python (*Python regius*). One more peculiar case was recorded, of a Marmoset (*Callithrix Jacchus*), which was rescued by Ponderosa Zoo and brought to the practice so it could be examined (Graphic 1).



Graphic 1 – Case distribution of animals presented to the practice by patient species, expressed in Fr (%)

Regarding the case distribution by clinical area (Table 1), the largest sum of patients observed were directed for surgical medicine (33.2%), followed by clinical medicine (30.6%), preventive medicine (21.4%), medical procedures (8.8%) and, finally, imaging procedures (6.1%). It is possible to declare, once more, that most cases belonged to canine patients, in all clinical areas, followed by felines and finally exotics, which encloses all of the remaining species.

It is noteworthy that there are more procedures (n=808) than patients seen (n=717), meaning that one patient might have been subject to different approaches during its stay in the practice.

Table 1 – Observed case distribution by clinical area and patient species

Clinical area	Fip Canine	Fip Feline	Fip Exotic	Fi	Fr (%)
Preventive Medicine	121	51	1	173	21.4
Clinical Medicine	144	86	17	247	30.6
Surgical Medicine	151	102	15	268	33.2
Imaging procedures	37	10	2	49	6.1
Medical procedures	43	15	13	71	8.8
Total	496	264	50	808	100.0

2. Preventive Medicine

When it comes to preventative medicine, the category mainly includes vaccination, microchipping and deworming (Table 2). Vaccination and deworming contribute to the well-being of humans and animals alike, by preventing the spread of disease, in the form of zoonosis, and by significantly reducing morbidity and mortality rates of infectious diseases (Moore & HogenEsch, 2010; Stull, Brophy & Weese, 2015). Vaccines work by stimulating the patients innate and adaptive immune response, with the latter offering protection against future exposure to the disease (Day, 2006; Moore & HogenEsch, 2010). They are classified, according to the Vaccination Guidelines Group (VGG), as core, non-core and not recommended. The core vaccines are essential and need to be administered to all animals so they can develop immunity. Non-core vaccines are only required in certain cases, depending on geographical location, local environment and lifestyle. Finally, vaccines labelled as not recommended are the ones that do not possess sufficient scientific evidence to justify their use (Day *et al.*, 2016).

Internal and external deworming, representing 10.4% of the procedures in this bracket, is done at Donaldson's Vets using milbemycin oxime and praziquantel to treat mixed infections by adult cestodes and nematodes in dogs and cats, and sarolaner or selamectin for the treatment of flea, tick, lice and mite infestations. Milbemycin oxime and praziquantel are approved for use in dogs over 500 grams of body weight and/or over two weeks of age, being then subsequently treated at four, eight and 12 weeks, then every three months, and cats over 500 grams of body weight and/or over six weeks of age, with the treatment then applied at eight and twelve weeks, then every three months (Milpro film-coated tablets for Cats and Kittens, 2016; Milpro film-coated tablets for Dogs and Puppies, 2016). Sarolaner is indicated for the treatment of puppies over eight weeks of age and/or dogs over 1.3 kg of body weight (Simparica chewable tablets for dogs, 2016), and selamectin is indicated for the treatment of kittens over eight weeks of age and/or cats over 1.25 kg of body weight, with both the medication given once every month (Stronghold Plus spot-on solution for cats, 2016).

Microchipping was the least recorded procedure, with only 5.2% (n=9) instances counted. According to the British legislation (*Get your dog or cat microchipped*, 2016), the microchip should be applied and the pet registered in the database as soon as eight weeks old, in the case of dogs,

and by the time they reach 20 weeks in the case of cats. However, unlike dogs, cats in England only need to be microchipped from 10th of June 2024.

Vaccination corresponds to the most common prophylactic procedure (84.4%), with a total of 146 vaccines administrated during the traineeship. At Donaldson's, Versican® and Versifel® (antigen vaccines with DHPPi/L4 lyophilisate and CRV, respectively), made by Zoetis UK Limited, in Springfield Drive, are the preferred vaccine brands, however other brands, like Nobivac® (antigen vaccine with DHP), produced by MSD Animal Health UK Limited, in Buckinghamshire, are available in case there is preference towards it or if the owner already started the pet's vaccination somewhere else. For dogs, the vaccination plan starts at six weeks of age, with an DHPPi/L4 vaccine, and it is divided into two doses with an interval of three to four weeks between them. Afterwards, DHP becomes a single dose vaccine administered every three years and Pi/L4 administered annually. This vaccine grants immunity against the distemper virus, canine adenovirus type 2, parvovirus, canine parainfluenza virus and four different strains of *Leptospira* spp. In opposition to Portugal, in the UK the rabies vaccine is not compulsory. However, in case the owner wants to travel with the pet to another country, it must be vaccinated at least 21 days before travelling. The primary-vaccination should be done at eight weeks, instead of six, with the second dose given three to four weeks later together with the rabies vaccine, but not before the puppy reaches twelve weeks *(*Versican Plus DHPPi/L4 lyophilisate and suspension for suspension for injection for dogs, 2016; Versiguard Rabies, 2016). For cats, the vaccination plan starts at nine weeks, divided into two doses given three to four weeks apart. From there, the vaccine should be given annually. The cat vaccine (CRV) concedes immunity against the feline parvovirus, feline rhinotracheitis virus (herpesvirus type 1) and feline calicivirus (Versifel CVR, 2016). The feline leukaemia virus vaccine follows the same scheme as the CRV, although, after the completion of the primary vaccination scheme, a single booster should be administered one year thereafter. Later, a single booster dose should be given once every three years (Versifel® FeLV suspension for injection for cats, 2015). This vaccination plan is in accordance with the Zoetis guidelines for optimal use of the vaccine, however it differs slightly from the World Small Animal Veterinary Association (WSAVA) vaccination guidelines, regarding puppy vaccine schedules.

Table 2 – Case distribution related to preventative medicine by procedure and patient species

Preventive Medicine	Fip Canine	Fip Feline	Fip Exotic	Fi	Fr (%)
Internal and external deworming	8	10	0	18	10.4
Microchip	7	2	0	9	5.2
Vaccination	106	39	1	146	84.4
Total	121	51	1	173	100.0

From the total vaccines recorded (n=146), only one vaccination scheme for the exotic, in this case, a rabbit, was observed. The vaccination plan for this species includes protection against myxomatosis and two strands of the rabbit (viral) haemorrhagic disease. Rabbits can be

vaccinated from five weeks of age and will require a second, separate vaccine against the second strain of rabbit haemorrhagic disease at 10 weeks of age. From there on, the vaccine becomes annual. This is in accordance with the Royal Society for the Prevention of Cruelty to Animals (RSPCA) recommendations for vaccines in this species (Rabbit vaccinations, 2019).

3. Clinical Medicine

The area of clinical medicine is divided into 16 divisions, that will be boarded individually, with a brief bibliographic review made on each sub-category. By analysing Table 3 it is possible to deduce that the clinical area of dermatology and allergology was the most prevalent one (20.2%), followed by orthopaedics (14.2%) and gastroenterology (10.5%). In the other end of the spectre, there are the areas of pulmonology, haematology and immunology (both with 1.6%), toxicology (1.2%) and cardiology (0.4%). Due to the reduced number in exotic animals seen in practice, this group represents fewer cases (n=17) than canine (n=144) and feline (n=86) patients.

Table 3 - Case distribution related to clinical medicine by specialty and patient species

Clinical Medicine	Fip Canine	Fip Feline	Fip Exotic	Fi	Fr (%)
Cardiology	1	0	0	1	0.4
Dermatology and allergology	36	13	1	50	20.2
Endocrinology	4	11	0	15	6.1
Gastroenterology	16	10	0	26	10.5
Haematology and immunology	4	0	0	4	1.6
Infectiology and parasitology	1	5	4	10	4.0
Neurology	12	1	1	14	5.7
Oncology	13	8	1	22	8.9
Ophthalmology	8	5	3	16	6.5
Orthopaedics	30	5	0	35	14.2
Pulmonology	1	2	1	4	1.6
Stomatology and odontology	1	9	1	11	4.5
Theriogenology	5	2	0	7	2.8
Toxicology	3	0	0	3	1.2
Traumatology and emergency	7	2	3	12	4.9
Uronephrology	2	13	2	17	6.9
Total	144	86	17	247	100.0

3.1. Cardiology

Cardiology was the lowest recorded reason for visiting practice, with only one case documented by the trainee. This can be attributed to the lack of cardiology specialists at the practice, being that most cases ended up being redirected to referral hospitals. The only cardiology case reported was a congestive heart failure (Table 4), which came in as an emergency case.

This case belonged to a King Charles Cavalier, which was brought to the hospital. The patient had a previous history of heart problems (mitral valve disease) and was already on a high dose of furosemide (2 mg/kg twice a day).

Table 4 – Case distribution related to cardiology by disorder and patient species

Cardiology	Fip Canine	Fip Feline	Fip Exotic	Fi	Fr (%)
Congestive heart failure	1	0	0	1	100
Total	1	0	0	1	100

3.2. Dermatology and Allergology

Dermatology and allergology represented 20.2% of the total case reports in clinical medicine analysed during the traineeship. The most common occurrence (Table 5) was external otitis, detected in 46% of the patients, followed by atopic dermatitis (12%), and cutaneous abscesses (10%). In this category, only one case was reported in exotics, as a leopard gecko had some stuck shed near its eye that needed careful removal. Most of the patients that had external otitis were dogs, especially Cocker Spaniels and Cocker mixes, due to their long ears and dense fur. The most common disease observed in cats was cutaneous abscesses, especially cat bite abscesses, as most of them have access to outdoors.

Table 5 - Case distribution related to dermatology and allergology by disorder and patient species

Dermatology and Allergology	Fip Canine	Fip Feline	Fip Exotic	Fi	Fr (%)
Atopic dermatitis	6	0	0	6	12
Cellulitis	1	0	0	1	2
Contact dermatitis	0	2	0	2	4
Cutaneous abscesses	0	5	0	5	10
External otitis	20	3	0	23	46
Flea allergy dermatitis	2	0	0	2	4
Food allergy dermatitis	1	2	0	3	6
Pododermatitis	2	0	0	2	4
Pyoderma	2	1	0	3	6
Skin laceration	2	0	0	2	4
Stuck shed	0	0	1	1	2
Total	36	13	1	50	100

3.3. Endocrinology

When it comes to endocrine disorders, feline patients have a higher number of cases (n=11), even though less cats were seen in comparison to dogs (Table 6). Consequently, the most prevalent of these diseases was hyperthyroidism, which represented 66.7% of the cases in this category, as it is the most common endocrinopathy in domestic cats. The remaining two diseases presented in hospital were hyperadrenocorticism (26.7%) and Diabetes *mellitus* (6.7%).

Table 6 – Case distribution related to endocrinology by disorder and patient species

Endocrinology	Fip Canine	Fip Feline	Fip Exotic	Fi	Fr (%)
Hyperadrenocorticism	4	0	0	4	26.7
Diabetes *mellitus*	0	1	0	1	6.7
Hyperthyroidism	0	10	0	10	66.7
Total	4	11	0	15	100.0

3.4. Stomatology and Odontology

Stomatology and odontology cases were also more commonly seen in cats (n=9) than in dogs (n=1) or exotics (n=1) whilst in practice. The majority stomatology and odontology cases are gingivitis (36.4%) followed by root abscesses (27.3%), periodontal disease (18.2%), chronic gingivostomatitis and malocclusion (both at 9.1%) (Table 7).

Table 7 - Case distribution related to stomatology and odontology by disorder and patient species

Stomatology and odontology	Fip Canine	Fip Feline	Fip Exotic	Fi	Fr (%)
Chronic gingivostomatitis	0	1	0	1	9.1
Gingivitis	0	4	0	4	36.4
Maloccluded teeth	0	0	1	1	9.1
Periodontal disease	0	2	0	2	18.2
Root abscess	1	2	0	3	27.3
Total	1	9	1	11	100.0

3.5. Gastroenterology

Gastroenterology being the third most common clinical medicine occurrence had a wide spread of cases present, with twelve different diagnoses made in twenty six different patients. However, despite having a wide variety of cases, it was not possible to identify any exotic species in this category (Table 8).

Table 8 - Case distribution related to gastroenterology by disorder and patient species

Gastroenterology	Fip Canine	Fip Feline	Fi	Fr (%)
Anal gland impaction or infection	2	0	2	7.7
Colitis	1	0	1	3.8
Faecaloma	0	2	2	7.7
Feline Gastrointestinal Eosinophilic Sclerosing Fibroplasia	0	1	1	3.8
Foreign body ingestion	2	0	2	7.7
Gastritis	2	1	3	11.5
Gastroenteritis	6	3	9	34.6
Inflammatory bowel disease	1	0	1	3.8
Intussusception	1	1	2	7.7
Pancreatitis	1	1	2	7.7

Rectal prolapse	0	1	1	3.8
Total	16	10	26	100.0

The most common occurrence was gastroenteritis (34.6%), followed by gastritis (11.5%), anal gland infection, faecaloma, foreign body injection, intussusception and pancreatitis (all at 7.7%). Colitis, feline gastrointestinal eosinophilic sclerosing fibroplasia (FGESF), inflammatory bowel disease, and renal prolapse (all at 3.8%) were the least seen cases. FGESF was only possible to diagnose through surgery (exploratory laparoscopy) and biopsy of the stomach.

3.6. Haematology and Immunology

Haematology and immunology cases were, like cardiology, scarce. However, four instances were reported, all in dogs, of three different diseases. From these, immune-mediated thrombocytopenia (IMTP) was found in two separate occasions (50%), lupus erythematosus and von Willebrand disease were both recorded once (25%) (Table 9). For this table, only animals that presented primary haematologic processes and/or coagulopathies were considered.

Table 9 - Case distribution related to haematology and immunology by disorder and patient species

Haematology and immunology	Fip Canine	Fi	Fr (%)
Immune-mediated thrombocytopenia	2	2	50
Lupus erythematosus	1	1	25
von Willebrand disease	1	1	25
Total	4	4	100

3.7. Infectiology and Parasitology

Infectiology and Parasitology are of upmost importance in the clinical area, as some diseases with zoonotic potential belong to this category. However, besides the *Toxascaris leonina* infection detected in one ferret, no pathogens with potential zoonotic threat were detected. From the total cases observed in this area (n=10) the majority were found in cats. With the highest frequency were three feline viral rhinotracheitis (feline herpesvirus type-1) cases (30%), followed by two sour crop (*Candida albicans*) infected chickens (20%), which were detected in the same coop, one feline infectious peritonitis (feline coronavirus) case, one kennel cough (*Bordetella bronchiseptica*) case, which was the only canine infectious disease detected, one cat with mycoplasmosis and one case of oxyurids infestation in a bearded dragon (10% each) (Table 10).

Table 10 - Case distribution related to infectiology and parasitology by disorder and patient species

Infectiology and parasitology	Fip Canine	Fip Feline	Fip Exotic	Fi	Fr (%)
Feline infectious peritonitis	0	1	0	1	10
Feline viral rhinotracheitis	0	3	0	3	30
Kennel cough	1	0	0	1	10
Mycoplasmosis	0	1	0	1	10

Oxyuridosis	0	0	1	1	10
Sour crop	0	0	2	2	20
Toxocariasis	0	0	1	1	10
Total	1	5	4	10	100

3.8. Uronephrology

Uronephrology cases were once more seen more in feline patient's rather than canine, despite the difference in the number of cases (Table 11). The most commonly presented case in this area is chronic kidney disease (41.2%; n=7), which is also one of the most common diseases identified in senior cats. Urinary tract infections (29.4%; n=5), non-obstructive idiopathic cystitis (11.8%; n=2), acute kidney injury (5.9%; n=1), urinary incontinence (5.9%; n=1) and urolithiasis (5.9%; n=1) were also detected.

Table 11 - Case distribution related to uronephrology by disorder and patient species

Uronephrology	Fip Canine	Fip Feline	Fip Exotic	Fi	Fr (%)
Acute kidney injury	0	1	0	1	5.9
Chronic kidney disease	1	6	0	7	41.2
Non-obstructive idiopathic cystitis	0	2	0	2	11.8
Urinary incontinence	1	0	0	1	5.9
Urinary tract infection	0	3	2	5	29.4
Urolithiasis	0	1	0	1	5.9
Total	2	13	2	17	100.0

3.9. Neurology

Neurology cases were more broadly detected in dogs than in other species (Table 12), with the most common being idiopathic epilepsy (57.1%), present in seven dogs and one cat. Moreover, a case of encephalomyelitis was discovered in a domestic chicken that was brought to the hospital after the owner found it unable to balance. The remaining cases, which involved canine cognitive dysfunction (7.1%; n=1), discal hernia (21.4%; n=3), two of which on the cervical region (C2-C3), and one in the thoracic region (T11-T12), and laryngeal paralysis (7.1%; n=1) were all found in dogs.

Table 12 - Case distribution related to neurology by disorder and patient species

Neurology	Fip Canine	Fip Feline	Fip Exotic	Fi	Fr (%)
Canine cognitive dysfunction	1	0	0	1	7.1
Discal hernia	3	0	0	3	21.4
Encephalomyelitis	0	0	1	1	7.1
Idiopathic epilepsy	7	1	0	8	57.1
Laryngeal paralysis	1	0	0	1	7.1
Total	12	1	1	14	100.0

3.10. Oncology

Donaldson's has a partnership with an independent veterinary oncology referral service, called Cancer Care for Pets, hence a lot of their cases pass through the practice before being redirected to them. As a consequence, twenty two oncology cases were reported, being the most prevalent alimentary lymphomas (31.8%; n=7) and lipomas (13.6%; n=3). Other neoplasia found included adrenal gland adenocarcinomas (4.5%; n=1), cutaneous histiocytoma (4.5%; n=1), hemangiosarcoma, both on the spleen (9.1%; n=2), mammary gland tumour (4.5%; n=1), cutaneous mas cell tumour (9.1%; n=2), osteosarcoma (9.1%; n=2), sarcoma, both on the front limbs (9.1%; n=2), and squamous cell carcinoma, on the nasal planum and lips of a cat (4.5%; n=1). The only exotic case reported was of an osteosarcoma in a bearded dragon (Table 13).

Table 13 - Case distribution related to oncology by disorder and patient species

Oncology	Fip Canine	Fip Feline	Fip Exotic	Fi	Fr (%)
Adrenal gland adenocarcinoma	1	0	0	1	4.5
Alimentary lymphoma	1	6	0	7	31.8
Cutaneous histiocytoma	1	0	0	1	4.5
Hemangiosarcoma	2	0	0	2	9.1
Lipoma	3	0	0	3	13.6
Mammary gland tumour	0	1	0	1	4.5
Mast cell tumour	2	0	0	2	9.1
Osteosarcoma	1	0	1	2	9.1
Sarcoma	2	0	0	2	9.1
Squamous cell carcinoma	0	1	0	1	4.5
Total	13	8	1	22	100.0

3.11. Ophthalmology

Once more, due to the lack of specialist in ophthalmology in Donaldson's, most cases seen are those of acute onset. Superficial corneal ulcers represent the majority of cases (56.25%) with a similar incidence in both feline and canine patients (four cases each). Six conjunctivitis cases (37.5%) and a retrobulbar abscess (6.25%) were also recorded (Table 14).

Table 14 - Case distribution related to ophthalmology by disorder and patient species

Ophthalmology	Fip Canine	Fip Feline	Fip Exotic	Fi	Fr (%)
Conjunctivitis	3	1	2	6	37.5
Retrobulbar abscess	1	0	0	1	6.25
Corneal ulcer	4	4	1	9	56.25
Total	8	5	3	16	100

3.12. Orthopaedics

Orthopaedic patients are found commonly at Donaldson's, as there are many veterinarians interested in the field. Out of the 35 total clinical cases, 25 were osteoarthritic

patients (71.4%), four had cruciate ligament rupture (11.4%), two had a fractured pelvis (5.7%), one had a fractured humerus (2.9%), one had a fracture in one of the digits (2.9%), and two had patellar luxation (5.7%) (Table 15). Most of the osteoarthritic patients started taking Librela® (bedinvetmab), a canine monoclonal antibody, or Solensia® (frunevetmab), a feline monoclonal antibody, both manufactured by Zoetis UK Limited. Injections were given subcutaneously once a month. These monoclonal antibodies target the nerve growth factor, inhibiting its signalling to the cells and providing pain relieve. The remaining were all directed to orthopaedic surgery.

Table 15 - Case distribution related to orthopedia by disorder and patient species

Orthopaedics		Fip Canine	Fip Feline	Fi	Fr (%)
Cruciate ligament rupture		4	0	4	11.4
Fracture	Pelvis	1	1	2	5.7
	Humerus	0	1	1	2.9
	Digit	1	0	1	2.9
Osteoarthritis		22	3	25	71.4
Patellar luxation		2	0	2	5.7
Total		30	5	35	100.0

3.13. Pulmonology

Respiratory diseases were not very commonly seen during the traineeships time period, as it only represents 1.6% of the total clinical medicine cases (Table 16). From the four cases recorded, two were cats with upper respiratory tract disease (one had chronic idiopathic rhinosinusitis, previously diagnosed in another consult, and another due to the inhalation of a foreign body), one was bronchopneumonia in a dog, and one was a rhinolith in a parakeet.

Table 16 - Case distribution related to pulmonology by disorder and patient species

Pulmonology	Fip Canine	Fip Feline	Fip Exotic	Fi	Fr (%)
Bronchopneumonia	1	0	0	1	25
Rhinolith	0	0	1	1	25
Upper respiratory tract disease	0	2	0	2	50
Total	1	2	1	4	100

3.14. Theriogenology

Theriogenology cases were an uncommon occurrence during the traineeship, with only seven cases seen. From these, five were identified in dogs and two in cats, which presented two cases of mastitis (28.6%), two pyometras (28.6%), two pseudo-pregnancies (28.6%), and a case of vulvar thrush, (14.3%), a common yeast infection by Candida albicans (Table 17).

Table 17 - Case distribution related to theriogenology by disorder and patient species

Theriogenology	Fip Canine	Fip Feline	Fi	Fr (%)
Mastitis	1	1	2	28.6

Pyometra	2	0	2	28.6
Pseudo-pregnancy	1	1	2	28.6
Vulvar thrush	1	0	1	14.3
Total	5	2	7	100.0

3.15. Toxicology

Admits due to the ingestion of toxics were the second least common reason for clinical intervention at the practice (Table 18). This can be due to an increase in awareness and availability of information for pet owners, allowing them to know about certain products and their possible negative effects to their pets. That being said, accidents still happen, as it was the case of both chocolate ingestion cases (66.7%), where the pets allegedly stole the candy when the tutor was away. The rodenticide ingestion (33.3%) ended up being a fatal case, as the owner only realized the dog had eaten the poison when the pet collapsed.

Table 18 - Case distribution related to toxicology by disorder and patient species

Toxicology	Fip Canine	Fi	Fr (%)
Chocolate ingestion	2	2	66.7
Rodenticide ingestion	1	1	33.3
Total	3	3	100

3.16. Traumatology and Emergency

The majority of cases presented in this category are secondary to trauma, with the exception of diabetic ketoacidosis and ischemic stroke, both representing 8.3% of these cases. The dog that presented with an haemoabdomen (8.3%) had a tumour in its spleen, hence making it more susceptible to rupture. The majority of cases in this category were bite wounds (41.7%; n=5), followed by aural haematoma (8.3%; n=1), polytraumatized (16.7%; n=2) and burns (8.3%; n=1) (Table 19). Although aural haematoma cases do not present danger to the animals' life, they are considered a trauma, hence they are included in this table. The diabetic ketoacidosis case came into the practice as an emergency case (collapsed and severely decompensated), and therefore also being included in this category.

Table 19 - Case distribution related to traumatology and emergency by disorder and patient species

Traumatology and emergency	Fip Canine	Fip Feline	Fip Exotic	Fi	Fr (%)
Aural haematoma	1	0	0	1	8.3
Bite wounds	4	0	1	5	41.7
Burns	0	0	1	1	8.3
Diabetic ketoacidosis	0	1	0	1	8.3
Haemoabdomen	1	0	0	1	8.3
Ischemic stroke	1	0	0	1	8.3
Polytraumatized	0	1	1	2	16.7
Total	7	2	3	12	100.0

4. Surgical Medicine

The area of surgical medicine is divided into six categories, depending on the type of surgery performed (Table 20). This is an important area at Donaldson's, as there are 268 procedures reported, which corresponds to 33.2% of the total case load. That being said, the majority of surgeries accompanied during the traineeship were soft tissue surgeries, which comprise 80.6% (n=216) of the total surgery count, followed by orthopaedic (9.7%; n=26), endosurgery (5.2%; n=14), dentistry (3.7%; n=10) and neurosurgery (0.7%; n=2). This distribution of cases also reflects the trainee's preferences for surgery performed. Furthermore, all surgeries happened during the morning period (from 08:30 am until 01:00 pm), hence not being possible to accompany equally all areas.

Table 20 - Case distribution related to surgical medicine by procedure and patient species

Surgical Medicine	Fip Canine	Fip Feline	Fip Exotic	Fi	Fr (%)
Dentistry	9	0	1	10	3.7
Endosurgery	14	0	0	14	5.2
Neurosurgery	2	0	0	2	0.7
Orthopaedic surgery	22	4	0	26	9.7
Soft tissue surgery	104	98	14	216	80.6
Total	151	102	15	268	100.0

4.1. Soft tissue surgery

Soft tissue surgery represented the majority of surgical procedures performed at Donaldson's Vets. This category includes elective surgeries (castration and ovariohysterectomy or ovariectomy), which are done routinely, which explains the higher number of cases in this category (Table 21).

The most common procedure was elective castration, with 99 reported cases (45.8%), followed by elective ovariohysterectomy or ovariectomy. These last two procedures were divided into midline and flank, as they comprise surgeries done in both bitches and queens, respectively. It is still noteworthy to know that at Donaldson's, routine cat spays are done by flank, instead of midline. However, there were two instances where that did not happen, as these were done due to the surgeon's preference for that approach. Cat spays were more common than dog spays, with 31 and 22 total cases reported, being that 26 were done by midline (12%) and 29 by flank (13.4%). The remaining procedures included: anal sacculectomy (0.9%; n=4), caesarean section (1.4%; n=3), inguinal cryptorchid castration (0.9%; n=2), cystotomy (0.9%; n=2), drainage of abscesses (0.5%; n=1), drained aural haematomas (1.4%; n=3), enterectomy (2.8%; n=6), enterotomy (1.9%; n=4), exploratory laparotomy (1.4%; n=3), abdominal and umbilical hernia repairs (0.5%; n=2), laryngeal tie-back (0.5%; n=1), liver lobectomy (0.5%; n=1), mastectomy (1.4%; n=3), nephrectomy (1.4%; n=3); ovariohysterectomy due to pyometra (1.9%; n=4),

splenectomy (0.9%; n=2), simple sutures (1.4%; n=3), surgical biopsies (5.6%; n=12), thyphlectomy (0.5%; n=1), total ear canal ablation (0.5%; n=1), urethropexy (0.5%; n=1), and wound debridement (0.5%; n=1). One can observe that more procedures were performed on cats than on dogs, despite there being fewer feline patients, and this is due to financial assistance and regional campaigns given to cat owners that have rescued or adopted stray cats and/or those in financial difficulties. The procedures done in exotic animals were all performed in ferrets that belong to the Prospect Ferret Rescue, as Donaldson's provides service to this institution that provides a safe refuge home to stray and unwanted ferrets.

It is also important to refer that there were more pyometra surgeries than the ones diagnosed in hospital, this due to the pyometra cases found in other clinics behind forwarded to the hospital for surgery.

Table 21 - Case distribution related to soft tissue surgery by procedure and patient species

Soft tissue surgery			Fip Canine	Fip Feline	Fip Exotic	Fi	Fr (%)
Anal sacculectomy			2	0	0	2	0.9
Abscess drainage			0	1	0	1	0.5
Aural haematoma drainage			3	0	0	3	1.4
Caesarean section			3	0	0	3	1.4
Castration	Inguinal cryptorchidism		2	0	0	2	0.9
	Elective		31	56	12	99	45.8
Cystotomy			2	0	0	2	0.9
Enterectomy			3	3	0	6	2.8
Enterotomy			4	0	0	4	1.9
Exploratory laparotomy			2	1	0	3	1.4
Hernia repair			0	1	0	1	0.5
Laryngeal Tie-Back			1	0	0	1	0.5
Liver Lobectomy			1	0	0	1	0.5
Mastectomy			1	2	0	3	1.4
Nephrectomy			1	2	0	3	1.4
Ovariohysterectomy or ovariectomy	Elective	Midline	22	2	2	26	12.0
		Flank	0	29	0	29	13.4
	Pyometra		4	0	0	4	1.9
Splenectomy			2	0	0	2	0.9
Simple suture			3	0	0	3	1.4
Surgical biopsy			11	1	0	12	5.6
Thyphlectomy			1	0	0	1	0.5
Total Ear Canal Ablation			1	0	0	1	0.5
Urethropexy			1	0	0	1	0.5
Wound debridement			3	0	0	3	1.4
Total			104	98	14	216	100

4.2. Dentistry

The dentistry service at the practice is usually divided between the branches, hence there are not many cases available at the hospital. That being said, no feline patients were observed by the author during the stipulated time period. In total, ten cases were observed, where tooth scale and polish represented the majority (60%; n=6) of these. Three tooth extractions (30%) and an ameloblastoma removal (10%) were also observed (Table 22).

Table 22 - Case distribution related to dentistry by procedure and patient species

Dentistry	Fip Canine	Fip Exotic	Fi	Fr (%)
Ameloblastoma removal	1	0	1	10
Tooth extraction	3	0	3	30
Tooth scale and polish	5	1	6	60
Total	9	1	10	100

4.3. Orthopaedic surgery

Orthopaedic surgery was, after soft tissue surgery, the second most predominant type of surgical procedure performed at the practice (Table 23). From these, the tibial plateau levelling osteotomy (TPLO) was by far the most common procedure, with twelve total cases recorded (46.2%), as this was the surgery of choice for the orthopaedic surgeons at Donaldson's for large breed canines. Tail amputations were the second most executed procedure, with five recorded instances (19.2%). A more complex surgery performed in a Labrador that required both proximal tibial osteotomy and TPLO to be performed, as the dog had both patella luxation, due to an over exaggerated proximal tibial arch, and cruciate ligament rupture.

Table 23 - Case distribution related to orthopaedic surgery by procedure and patient species

Orthopaedic surgery		Fip Canine	Fip Feline	Fi	Fr (%)
Amputation	digit	1	0	1	3.8
	limb	1	1	2	7.7
	tail	4	1	5	19.2
Proximal tibial osteotomy		1	0	1	3.8
Fabellotibial suture		2	0	2	7.7
Fracture repair		0	1	1	3.8
Tarsal arthrodesis		0	1	1	3.8
Tibial Plateau Levelling Osteotomy		12	0	12	46.2
Tibial Tuberosity Advancement		1	0	1	3.8
Total		22	4	26	100

4.4. Endosurgery

Endosurgery at Donaldson's is becoming a more explored alternative to traditional surgery for routine procedures, such as ovariectomy and abdominal cryptorchid orchiectomy, due to their advantages. These surgeries are less invasive and tend to have a faster recovery time then their "traditional" counterparts. However, they are also more expensive to perform, hence not many owners are appealed to this method. From the recorded procedures, the vast majority were ovariectomies (92.9%; n=13), with only one case of cryptorchid orchiectomy (7.1%) performed at the hospital (Table 24).

Table 24 - Case distribution related to endosurgery by procedure and patient species

Endosurgery	Fip dogs	Fi	Fr (%)
Abdominal cryptorchid orchiectomy	1	1	7.1
Ovariectomy	13	13	92.9
Total	14	14	100

4.5. Neurosurgery

Table 25 refers to neurosurgery, which includes only two ventral slot procedures. This surgical specialty was only performed by one surgeon in the practice, being most of them redirected to referral hospitals. These cases were both diagnosed in hospital through computed tomography.

Table 25 - Case distribution related to neurosurgery by procedure and patient species

Neurosurgery	Fip Canine	Fi	Fr (%)
Ventral slot surgery	2	2	100
Total	2	2	100

5. Imaging Procedures

Table 26 consists of all of the imaging procedures performed at the hospital. It is worth noting that these procedures were conducted at the same time as surgeries, hence they might be underestimated in this table. Radiography (36.7%; n=18) and ultrasonography (38.8%; n=19) are the most commonly used diagnostic imaging tools used at the hospital, due to their relatively fast and easy handling, together with their low cost, at least when compared with other, more advanced imaging procedures. Computed tomography was also fairly used (18.4%; n=9), mostly when it proved to be more advantageous than the remaining, and when Cancer Care for Pets required its use to locate satellite lymph nodes or metastasis on their patients for staging. It would be expected that more cases would have been seen with this procedure. However, the scanning machine suffered a malfunction and was out of commission for a while.

Table 26 - Case distribution related to the imaging procedures performed, regarding their type and patient species

Imaging Procedures	Fip Canine	Fip Feline	Fip Exotic	Fi	Fr (%)
Computed Tomography	7	2	0	9	18.4
Echocardiography	1	0	0	1	2.0
Echography	14	4	1	19	38.8
Endoscopy	0	1	0	1	2.0
Radiographs	14	3	1	18	36.7
Ultrasound-guided fine needle aspiration	1	0	0	1	2.0
Total	37	10	2	49	100

6. Medical Procedures

The medical procedures presented in this category (Table 27) all are either part of complementary diagnostic procedures or part of a treatment procedure for a specific disease or condition. The most commonly performed procedure was euthanasia, with 39 registered cases (54.9%), and there are a few reasons for this. First and foremost, a lot of patients that underwent euthanasia were either chronically ill and/or had a sudden (acute) onset that made the owner make this decision. An example of this are the idiopathic epilepsy cases, were three out of seven pets ended up being euthanised as a result. Another factor that favoured the decision to euthanise were the owner's funds and availability to actively participate in the pet's treatment. Moreover, there is a phenomenon in the United Kingdom around the holiday season (Christmas and Boxing Day), where there is a marked increase in euthanasia rates. Most pet owners nowadays see pets as an integral part of their families, consequently holding off on euthanasia until after the holidays. However, most of the times this is not possible, which leads to an emergency visit to the hospital, where euthanasia is performed.

Besides this, other procedures were executed, with the most common being fine needle aspirations (7.0%; n=5) as a means of diagnosis, emesis (5.6%; n=4) in dogs that ate foreign bodies, and infected/broken nails clipping and removal (5.6%; n=4).

Intravenous catheterization and blood sampling of patients were not recorded, as they are procedures done multiple times a day, recurrently, meaning it would be impractical to record all of them.

Table 27 - Case distribution related to the medical procedures performed, regarding their type and patient species

Medical Procedures	Fip Canine	Fip Feline	Fip Exotic	Fi	Fr (%)
Anal gland expression	3	0	0	3	4.2
Chemical abortion	2	0	0	2	2.8
Chemical castration	1	0	0	1	1.4
Chemical spay	0	0	1	1	1.4
Chemotherapy	2	0	0	2	2.8
Cryotherapy	2	0	0	2	2.8

Cystocentesis	0	1	0	1	1.4
Emesis induction	4	0	0	4	5.6
Enema	0	1	0	1	1.4
Euthanasia	19	11	9	39	54.9
Fine needle aspiration	2	1	2	5	7.0
Immunotherapy	2	0	0	2	2.8
Nasal swab	1	0	0	1	1.4
Pull nail	4	0	0	4	5.6
Shed removal	0	0	1	1	1.4
Suture removal	1	0	0	1	1.4
Urinary catheterization	0	1	0	1	1.4
Total	43	15	13	71	100

III. Monograph – Feline Chronic Kidney Disease

1. Introduction

Chronic kidney disease (CKD) is regarded as one of the most common metabolic disease of domestic cats (Brown *et al.*, 2016; Chen *et al.*, 2020). It is defined as the presence of any structural and/or functional abnormality of one or both kidneys that have been present for an extended period, usually no less than three months (Machado *et al.*, 2022; Polzin, 2011).

Although CKD occurs in all ages, it is more prevalent in older cats, affecting up to 71% of cats over 15 years (Schauf *et al.*, 2021). As a result of its high prevalence, CKD is also one of the most common causes of mortality in elderly cats (Sparkes *et al.*, 2016).

According to the International Renal Interest Society (IRIS), this disease can be classified into four stages, based on the levels of serum creatinine and symmetric dimethylarginine (SDMA), and further substaged depending on the presence of proteinuria and blood pressure measurements (*IRIS Kidney - Guidelines - IRIS Staging of CKD*, 2023).

As the name implies, CKD is a progressive disease, where the rate of progression varies from patient to patient. Unfortunately this condition is uncurable, since it is mostly detected when kidney function is heavily impaired and the lesions tend to be irreversible, with treatment being therefore, focused on controlling the disease rather than curing it. (Schauf *et al.*, 2021).

2. Kidney Anatomy

The kidneys are situated in the retroperitoneal space against the dorsal abdominal wall laying within a splitting of the sublumbar fascia, which also holds considerable fat that protects them against distorting pressures from the other organs (Singh & Dyce, 2018). Together with the ureters, bladder, and urethra, they form the urinary tract.

The right kidney contacts cranially with the caudate process of the caudate lobe of the liver, medially with the right adrenal gland and caudal vena cava, ventrally with the pancreas and

liver, and laterally with the last rib and abdominal wall. In turn, the left kidney lies bellow the second to fourth lumbar vertebrae, about half a kidney's length lower than the right one, contacting with the spleen cranially, the abdominal wall laterally, and the descending colon ventrally. Both kidneys are held in their respective positions by subperitoneal connective tissue and are protected by an outer fibrous capsule. When compared with other domestic species, the cat's kidneys are more mobile, especially the left one, in addition to being readily palpable through abdominal palpation (Breshears & Confer, 2017; Fossum, 2018).

2.1. Macroscopic anatomy

Macroscopically, the cat's kidneys are relatively large when compared with other domestic species and have a distinctive look. They assume a more rounded shape, instead of the traditional "bean" shape, with the presence of capsular veins converging over their surface towards the hilus. They have a smooth surface, except for an indentation, called the renal hilus, that leads to a concealed space inside the kidney named the renal sinus, which includes the renal pelvis, the vessels and nerves that irrigate and innervate the organ. The internal anatomy of the kidneys (Figure 1), called the renal parenchyma, is divided into two parts: cortex and medulla (König & Liebich, 2016; Fossum, 2018). The cortex, the outer layer of the parenchyma, has a reddish brown colour and a finely granular appearance, except in adult cats where it assumes a more yellowish colour due to the large lipid content of the tubular epithelial cells. The inner layer of the parenchyma (i.e., medulla) consists of a dark purplish zone from which the medullary rays extend into the cortex, and a pale, greyish red zone, with striations extending to the renal sinus. In addition, in the cats' medulla the renal pyramids are all fused, assuming a unilobar or unipyramidal structure, where the pyramid's base is capped by the cortex and its apex points towards the sinus, forming a single papilla that fits into a cup-like expansion of the sinus called the calix (Breshears & Confer, 2017; Singh & Dyce, 2018).

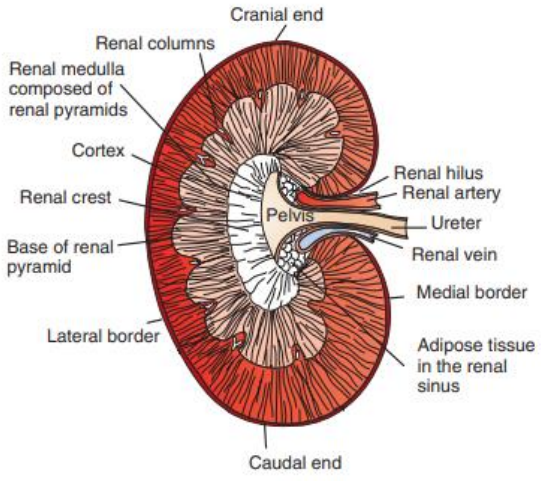


Figure 1 - Dorsal section of the kidney. Source: (Breshears & Confer, 2017)

2.2. Microscopic anatomy

Microscopically the kidney can be divided into four structural units: the renal corpuscle, which comprises the glomerulus and Bowman's capsule; tubules; interstitium; and vasculature (Breshears & Confer, 2017).

2.2.1. Kidney vascularisation and innervation

Each kidney is supplied by a branch from the aorta, called the renal artery (Figure 2). After entering the kidney this artery divides into several interlobular arteries that run along the corticomedullary junction of the renal pyramids and give origin to branches that curve over the bases of the pyramids called the arcuate arteries. These, in turn, give rise to the interlobular arteries that supply the lobules, divisions in the cortex caused by the medullary rays. The afferent glomerular arterioles arise from the interlobular arteries, enter the renal corpuscle, forming a network of capillaries, and exit the vascular pole as efferent glomerular arterioles (Pollak *et al.*, 2014). These efferent arterioles bring blood to an extensive network of capillaries, known as the peritubular capillary network, which irrigates the kidney's tubular system. After supplying blood to the tubules, these capillaries then drain into the interlobular vein. It is worthy to note that the blood flow in the vessels that irrigate the tubules is contrary to the direction of the urine flow (König & Liebich, 2016; Breshears & Confer, 2017; Singh & Dyce, 2018). On the other hand, the veins that ultimately lead to the caudal vena cava are satellites, which means they accompany the arteries until they merge into the renal vein and exit the kidney (König & Liebich, 2016; Singh & Dyce, 2018).

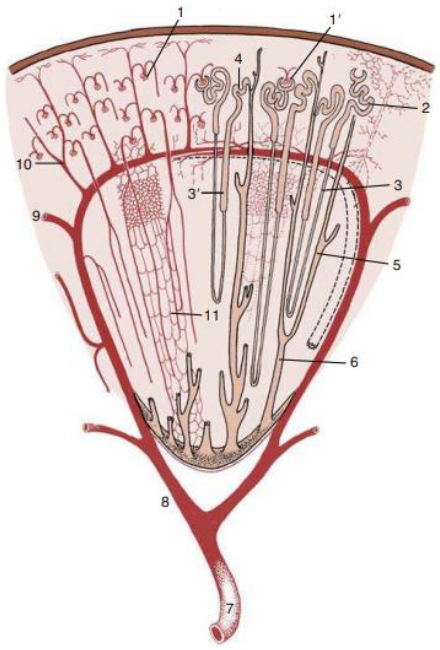


Figure 2 – Representation of a kidney lobe. 1, Glomerulus; 1', Renal Corpuscle; 2, proximal convoluted tubule; 3, descending limb of nephron; 3', ascending limb; 4, distal convoluted tubule; 5, collecting tubule;

6, papillary duct; 7, renal artery; 8, interlobar artery; 9, arcuate artery; 10, interlobular artery; 11, capillary plexus. Source: (Singh & Dyce, 2018)

The renal innervation occurs through the celiacomesenteric plexus, whose nervous fibres travel alongside the arteries that supply the kidney. The sympathetic nerves from the plexus form synapses with the celiac ganglia, mesenteric ganglia and aorticorenal ganglia, which are within the peripheral parts of the plexus. The parasympathetic innervation is ensured by a branch of the vagus nerve (König & Liebich, 2016; Singh & Dyce, 2018).

2.2.2. Glomerulus and uriniferous tubules

The uriniferous tubule is a term used to describe the renal corpuscle, which consists of the Bowman's capsule and the glomerulus, and the tubular system of the kidney, that includes the proximal tubule, loop of Henle, distal tubule and collecting ducts. The latter are enveloped in the interstitium.

The Bowman's capsule is a cup-shaped membranous sac that encloses the glomerulus without contacting with it, leaving a space in between them called uriniferous space or Bowman's space. The capsule's surface is lined with parietal epithelial cells, which resemble squamous epithelium (Breshears & Confer, 2017).

The glomerulus is a complex cluster of fenestrated endothelial capillaries held together by the mesangium, a structure of cells in a glycoprotein matrix secreted by the mesangial cells, which are capable of synthesizing collagen as well as secreting inflammatory mediators (Breshears & Confer, 2017). This structure is capable of filtration thanks to a barrier formed by the glomerular basement membrane (GBM), or *basal lamina*, the fenestrated endothelium of the capillaries and the pedicles of the podocytes. The GBM is composed of layers or laminas, having a central one, the *lamina densa*, which is thick and dense, and a thinner and electron-lucent layer covering it, the *lamina rara*, which is divided into *lamina rara interna* and *lamina rara externa*. The GBM has a network of type IV collagen, forming a porous infrastructure that, together with negatively charged glycoproteins that overly the podocytes, such as laminin and acidic proteoglycans, form the complete structure of the membrane and allow for a charge differential to be formed (Breshears & Confer, 2017). Hence, the laminas of the GBM have different electron density, being the *lamina densa* more resistant to the passage of electrons and the *lamina rara* less resistant (Verlander, 2020). The podocytes are part of the visceral epithelium of Bowman's capsule, are responsible for synthesising the components of the basement membrane and have cytoplasmatic processes embedded in the *lamina rara externa* that interdigitate with one another, allowing the formation of filtration slits between them. The slits are also composed of nephrin, which controls the slit size by its connection to the actin in podocytes (Breshears & Confer, 2017). Therefore, filtration occurs based on molecule size and electrical charge, being both possible because of the fenestrated endothelium of the capillary walls, the GBM formed with type IV collagen and anionic glycoproteins, and the filtration slits of the epithelium. Capillary pressure also aids the filtration process (Breshears & Confer, 2017).

The loop of Henle, located between both proximal and distal tubules, is divided into a descending, whose wall is thin and permeable, and an ascending limb, which initially still presents a thin, permeable wall, and then transitions to a thick, impermeable wall. The wall of the proximal tubule is lined with columnar epithelial cells that have microvilli, which allows for an increased area of surface absorption. The distal tubules, collecting tubules and the loop of Henle all have cuboidal epithelial cells, which help, through absorptive and secretory activities, to concentrate the urine (Breshears & Confer, 2017; Singh & Dyce, 2018).

The interstitium is composed of fibroblasts, connective tissue, and extracellular matrix that secretes glycosaminoglycans. This secretion increases with age and ischemic damage. The cells here located are also responsible for the local production of prostaglandins (Breshears & Confer, 2017; Singh & Dyce, 2018).

3. Kidney Physiology

3.1. Glomerular filtration

The kidney's primary role is to maintain homeostasis. The kidneys accomplish this goal by performing a diversity of functions such as eliminating metabolic wastes in the urine; acid-base regulation, mostly by reabsorption of bicarbonate from the glomerular filtrate; conservation of water through reabsorption; maintenance of normal extracellular potassium ion concentrations, by passive reabsorption; and control the endocrine function of three hormone axis, being these erythropoietin, vitamin D and renin-angiotensin-aldosterone system (RAAS). The kidneys are therefore capable of responding to water, electrolyte, and acid-base disturbances by altering each specific rate of reabsorption or secretion of these substances (Breshears & Confer, 2017; Verlander, 2020).

Blood begins its filtration process in the glomerulus. Here, the blood filtration starts thanks to the fenestrated epithelium of the capillary walls, laminin, polyanionic proteoglycans, fibronectin, enactin and other glycoproteins, allowing for a size and charge-dependent filtration. The only molecules allowed to pass through to the uriniferous/Bowman's space are water, proteins <70 kDa and small solutes (Breshears & Confer, 2017), whereas all cellular components and plasma proteins with the size of albumin or larger are retained in blood. The electrical charge of a molecule will also affect its rate of filtration, being the cationic (positively charged) form, more freely filtered than its neutral or anionic (negatively charged) form. This charge-dependent filtration is created by the strongly negatively charged endothelial surface layer (ESL) of the glomerular capillary walls and with the contribution of negatively charged glycoproteins incorporated in the GBM. These negative charges will repel then the negatively charged plasma proteins and inhibit their passage (Verlander, 2020). After filtration, all that remains is a fluid nearly identical to plasma called the glomerular filtrate. The rate of filtrate formed, also known as glomerular filtration rate (GFR), is

used clinically to determine renal function and it is expressed as millimetres of glomerular filtrate formed per minute per kilogram of body weight (mL/min/kg) (Breshears & Confer, 2017).

GFR is normally maintained at a relatively constant level despite changes in systemic blood pressure and renal blood flow, staying within the physiological range due to renal modulation of blood pressure and intravascular volume throughout the body, all thanks to humoral factors, in particular the renin-angiotensin-aldosterone system, and intrinsic control of renal blood flow, glomerular capillary pressure and the filtration coefficient, which is mediated by the myogenic reflex and the tubuloglomerular feedback, being these autoregulatory systems that control the blood flow resistance in the afferent and efferent arterioles. The filtration coefficient is the product of the filtration barrier permeability and its surface area (Verlander, 2020). The GFR can then be determined by two main factors, the mean net filtration pressure, and the filtration coefficient. The first is dependent on the glomerular capillary wall, which creates a barrier to the forces opposing and favouring filtration. The latter is the hydrostatic pressure of the blood within the capillaries and the oncotic pressure of the fluid that's filtrated into Bowman's space. However, the oncotic pressure is usually irrelevant since there are no high molecular weight proteins in this filtrate. The forces opposing filtration are the plasma oncotic pressure in the capillaries and the hydrostatic pressure inside Bowman's space (Verlander, 2020).

3.2. Regulation of vascular tone and glomerular filtration

Besides filtration, the glomerulus also regulates blood pressure, through the secretion of vasopressor agents and/or hormones, regulation of tubular metabolism, removal of macromolecules from circulation, and regulation of peritubular blood flow. A crucial part of the fulfilment of these functions is the juxtaglomerular apparatus, which includes: an afferent arteriole, an efferent arteriole, the *macula densa* and the extraglomerular mesangium. The smooth muscle of the afferent arteriole is modified, forming myoepithelial cells called granular extraglomerular mesangial cells, that secret renin, whose production is stimulated in response to decreased renal perfusion due to, most commonly, systemic hypotension or extracellular fluid volume depletion. These changes in perfusion are detected by baroreceptors in the afferent arteriole. Other causes of renin production stimulation are variations in sodium chloride (NaCl) levels and changes in sympathetic nerve activity. Renin will act on the circulating angiotensinogen produced in the liver, converting it to angiotensin I, which is then transformed into a more active form, angiotensin II, by angiotensin-converting enzymes (ACE) from the vascular endothelium of the lung (Verlander, 2020; Sula & Lane, 2022).

3.2.1. Intrarenal regulation

The renin-angiotensin system (RAS) can also be activated solely within the kidney for the regulation of intrarenal hemodynamic, glomerular filtration and permeability, by the production of not only renin and prorenin but also of angiotensinogen and ACE in the interstitial capillary endothelium and proximal tubule. The activation of the intrarenal RAAS may occur in concert with

or independently of the systemic renin-angiotensin system, however, if its activation is inappropriate it may contribute to a pathologic condition (Verlander, 2020; Lawson & Jepson, 2021).

The activated form of angiotensin I (i.e., angiotensin II) is a potent vasoconstrictor that directly increases systemic blood pressure and renal perfusion pressure, while equally decreasing renal blood flow as well as the filtration coefficient by increasing salt and water retention, intravascular volume and vascular resistance. To achieve this, angiotensin II not only promotes sodium reabsorption from the tubule fluid into the blood stream, but also stimulates the release of aldosterone and vasopressin from the adrenal gland and the pituitary respectively. These further enhance reabsorption of sodium, chloride, and water from the kidney tubules to the blood stream, which consequently increases renal perfusion pressure and extracellular volume. This effect, together with the activation angiotensin II type 1 receptors (AT1) by elevated plasma angiotensin II, causes a negative feedback mechanism that helps to maintain GFR and renal perfusion within the physiological range by supressing the release of renin (Figure 3) (Verlander, 2020).

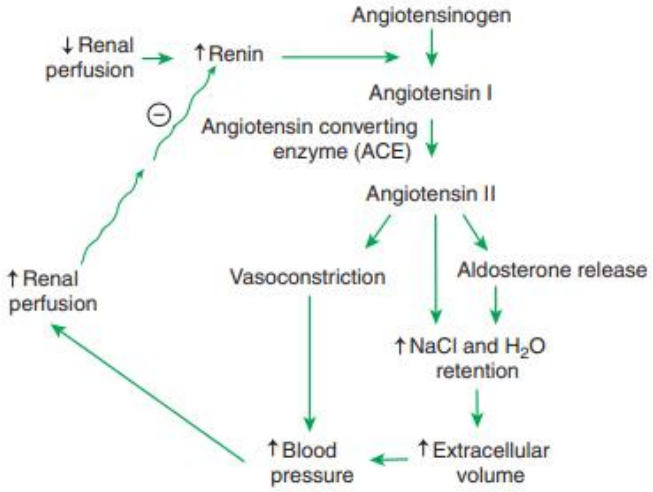


Figure 3 - Renin-angiotensin-aldosterone system. Source: (Verlander, 2020)

Overall, there are two systems that directly control glomerular capillary perfusion: the myogenic reflex and tubuloglomerular feedback. Both are autoregulatory mechanisms, but the first is initiated by changes in glomerular perfusion, whereas the latter is triggered by changes in sodium chloride delivery to the distal nephron (Verlander, 2020). The myogenic reflex regulates renal blood flow and GFR by constricting the afferent arteriole and other preglomerular vessels, when vessel distention is detected by stretch receptors located in the arterial walls, due to increased perfusion pressure. Conversely, arteriolar dilation occurs after wall tension reduces, thus reducing resistance to the blood flow. These changes in pressure help maintain GFR and renal blood flow constant despite changes in arterial blood pressure (Verlander, 2020). In turn, the tubuloglomerular feedback coordinates GFR with tubule transport capacity, which is ensured by the *macula densa*.

The *macula densa*, a distal portion of the thick ascending limb of the loop of Henle, is situated adjacent to the glomerulus on the extraglomerular mesangial region. As the GFR increases, the fluid flow in the tubules and sodium chloride concentrations also increase in the *macula densa*. Here, an apical uptake of NaCl via the sodium (Na) – potassium (K) – chloride (Cl) cotransporter (NKCC2) causes depolarization of cells and release of adenosine triphosphate (ATP), which is later degraded to adenosine. Afterwards, through signalling mediated by the mesangial cells, ATP and adenosine will affect GFR, causing vasoconstriction of the afferent arteriole and thereby increasing resistance to blood flow and reducing glomerular capillary perfusion pressure and glomerular filtration. Besides local effects, the activation of *macula densa* will also have systemic effects on intravascular volume, through the suppression of renin release, due to angiotensin II and aldosterone, and retainment of salt and water (Briggs *et al.*, 1991; Verlander, 2020). Although the main effect is to vasoconstrict the afferent arteriole, the increased delivery of NaCl to the distal nephron also stimulates the production of vasodilatory agents by *macula densa* cells, which include carbon monoxide (CO), nitric oxide (NO) and prostaglandin E_2 (PGE_2). The last two blunt the effects of the tubuloglomerular feedback so it does not cause excessive reductions in GFR (Verlander, 2020). There still exists a connecting segment, downstream of the *macula densa* and preglomerular arterioles, that was more recently discovered, called the connecting tubule-glomerular feedback system, which, like the tubuloglomerular feedback, is triggered by increased sodium chloride delivery in the renal tubules, but, unlike the latter, it acts to reduce afferent arteriolar tone by releasing vasodilators, PGE_2 and epoxyeicosatrienoic acids (Verlander, 2020).

Besides the mechanisms mentioned previously, the endothelium itself can contribute to the control of local renal vascular tone, producing its own constricting and dilating factors. One of the endothelium-derived relaxing factors, NO, holds significant importance in preventing renal damage by quenching reactive oxygen species, thus inhibiting intrarenal vasoconstriction, glomerular hypertension, mesangial cell proliferation and mesangial matrix production (Verlander, 2020).

The kidneys mechanisms for regulating vascular tone and glomerular filtration all interact with one another to maintain balance within these regulations. An example of these interactions is the effect of the vasoconstrictor angiotensin II, which can stimulate the release of endothelin that subsequently promote the release of NO and PGE_2, which are vasodilators. At the same time angiotensin II inhibits and enhances cyclooxygenase-2 (COX-2) expression and PGE_2 production, depending on which subtype of angiotensin receptor is activated (Verlander, 2020).

3.2.2. Systemic regulation

All the previously talked mechanism are intrarenal, nevertheless systemic factors can also contribute to changes in GFR through changes in blood volume and vessel tone. As mentioned before, angiotensin II promotes the release of vasopressin and aldosterone, enhancing water and NaCl reabsorption, which consequently increases blood volume. From the

cardiac atria, atrial natriuretic peptides can promote both diuresis (i.e., loss of water) and natriuresis (i.e., loss of sodium), thereby reducing blood volume (Lalor *et al.*, 2009; Verlander, 2020).

By reducing and redistributing renal blood flow there are also other factors able to affect vessel tone, including: circulating catecholamines, which increase blood pressure; and beta and alpha-adrenergic stimulation, that can activate the RAAS and promote renal vasoconstriction. Likewise, vasoconstrictors can also affect GFR (Verlander, 2020).

3.3. Renal tubules

The renal tubules have the role of selectively reabsorbing filtered substances, like glucose, sodium, and water, back into the bloodstream, as it travels through the tubule lumen, adapting to the needs and variations within the body. The proximal tubule has two pathways of reabsorption: the transcellular pathway and the paracellular pathway (Verlander, 2020).

The transcellular pathway transports substances largely by carrier-mediated transport through the apical plasma membrane, which possesses a brush border, composed of microvilli, cytoplasm and basolateral plasma membrane, into the interstitial fluid located between the capillary and epithelial cells. The apical plasma membrane also has epithelial infolds that improve surface area, which in turn, together with the brush border, allow for increased exposure for the luminal and interstitial fluids, facilitating transport (Verlander, 2020).

The paracellular pathway transports substances from the tubule across the *zonula occludens*, which is a permeable structure in the proximal tubule's cells that connects to claudin proteins, allowing the passive diffusion of certain solutes and water to the lateral intercellular space, which is thought to communicate with the interstitial fluid and be taken up by the peritubular capillary. This is possible due to the high oncotic pressure and low hydrostatic pressure on the peritubular vessels, which allows the interstitial fluid to go into the bloodstream. Reabsorption of solutes can happen due to different mechanisms, including primary active transport, passive diffusion, carrier-mediated secondary active transport and solvent drag (Verlander, 2020).

3.3.1. Proximal convoluted tubule

In the proximal tubule the most common out of these transport methods is transport mediated by the sodium-potassium-adenosine triphosphatase (Na-K-ATPase) pump, which allows active extrusion of three sodium ions (Na^+) from the cell, exchanging them for two potassium ions (K^+). In opposition, the potassium ions pass through K channels, creating an intracellular negative charge, in comparison with the extracellular space, which in turn increases sodium ion uptake through cotransport or countertransport. Specific sodium-dependent cotransporters for other molecules, like glucose, amino acids, phosphate, calcium, sulphate and citrate are mediated by secondary active transport. Besides fluctuations in plasma concentration, other systemic factors increase or decrease the uptake of these electrolytes (Verlander, 2020).

Bicarbonate ion (HCO_3^-) reabsorption is indirectly driven by the Na gradient by counter transporting hydrogen ions (H^+) across the apical plasma membrane through a Na^+/H^+ exchanger (Figure 4). The secreted H^+ combines with filtered HCO_3^- forming water (H_2O) and carbon dioxide (CO_2), thanks to the enzyme carbonic anhydrase located adjacent to the apical plasma membrane in the proximal tubule. CO_2 then enters the cells and interacts again with the carbonic anhydrase in the cytoplasm, leading to hydroxylation with hydroxyl ions (OH^-) donated from H_2O, forming HCO_3^- and H^+ inside the cell. HCO_3^- then goes through the basolateral membrane through a cotransporter, while the H^+ is transported into the tubule (Verlander, 2020).

Chloride ion (Cl^-) reabsorption also occurs in the proximal tubules, indirectly by the Na-K-ATPase pump, by both paracellular and transcellular routes. As other solutes and H_2O are being reabsorbed the concentration of Cl^- rises inside of the tubules reaching a chemical gradient. Also, as Na^+ uptake exceeds that of anions, it creates a positive charge inside the bloodstream, creating an electrical gradient favouring anion reabsorption. Passive, paracellular diffusion of Cl^- occurs through the *zonula occludens*, while transcellular transfer occurs due to the electrical and chemical gradients, established by Na-K-ATPase activity. Transport through the apical and basolateral plasma membranes occurs due to cotransporters (Verlander, 2020).

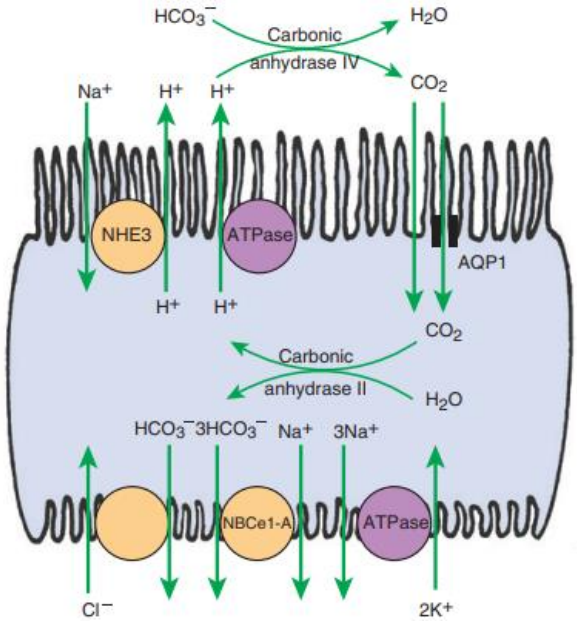


Figure 4 - Bicarbonate reabsorption cycle. Source: (Verlander, 2020)

In the distal portion of the proximal tubule, the transmembrane reabsorption of Na^+ will occur predominantly from NaCl uptake facilitated by coordinated action of Na^+ and Cl^- coupled transporters, where Cl^-/OH^- and Na^+/H^+ exchanges happen as well and allow the apical absorption of NaCl and the formation of H_2O in the tubule. Moreover, Na^+ will passively diffuse through the paracellular pathway due to the chemical and electrical gradient of Cl^- that was reabsorbed in previous portions of the tubule (Verlander, 2020).

Besides solutes and water, the proximal tubule also reabsorbs peptides and low molecular weight proteins. The first are degraded into amino acids by the peptidases located in the brush border and reabsorbed by cotransport with Na^+ or, if they are short-chained, they can be cotransport with H^+ through specific transporters in the brush border and degraded by intracellular peptidases (Verlander, 2020). The latter include insulin, glucagon, parathyroid hormone (PTH), and others, which are reabsorbed by receptor mediated endocytosis. The protein bind to a receptor, megalin or cubilin, in the apical plasma membrane and are endocytosed, being then delivered to the lysosomes which degrade the proteins and transport the amino acid end-products into the interstitial fluid, returning then to the blood (Verlander, 2020). Moreover, the proximal tubule also excretes a wide variety of organic ions, including endogenous waste, like bile salts, oxalate, urate, creatinine and prostaglandins, exogenous drugs (i.e., antibiotics, diuretics, antihypertensives, and some analgesic agents) and toxics (for instance, paraquat). These are usually protein bound, and thus poorly filtered in the glomerulus, needing than to be cleared by the proximal tubule, which is done by basolateral uptake from the blood and apical secretion into the circulation using specific transporters (Verlander, 2020).

3.3.2. Thick ascending limb, and distal convoluted tubule

The loop of Henle, a long U-shaped portion of the tubule, located distally to the proximal tubule, is divided into two portions: the thin limb and the thick ascending limb. The thin limb is a flat epithelium section with almost no membranous infolding or mitochondria, which means active transport of solutes is close to non-existent. Despite this, it allows for water and some solute reabsorption due to the special orientation in the medulla. Continuing down its path, the flat epithelium of the thin limb will then abruptly transition into the thick ascending limb with cuboidal epithelium, which contains a high number of mitochondria and basolateral plasma infoldings, responsible for active solute transport. After such portion, the cuboidal epithelium becomes taller, with a dense mitochondrial array, forming what is defined as the distal convoluted tubule (Verlander, 2020).

Regarding their function, the main mechanism of solute reabsorption, for both thick ascending limb and distal convoluted tubule, is also the Na-K-ATPase pump, present in the basolateral plasma membrane. Likewise, both have similar functions (Verlander, 2020).

In opposition to the proximal tubule, the thigh junctions and plasma membrane of the cuboidal epithelium are impermeable to water, which means that after reabsorption of Na^+, Cl^-, calcium ions (Ca^{2+}) and magnesium ions (Mg^{2+}), the tubule fluid will be hypotonic or diluted. This dilution happens regardless of the systemic fluid volume status and prevents blood plasma from becoming hypotonic when water intake is high. Furthermore, such process creates a hypertonic medullary interstitium, essential for urine concentration and water conservation, when the filtrated fluid reaches the collecting ducts (Verlander, 2020).

Specifically in the thick ascending limb, the Na^+ electrochemical gradient drives ion uptake through apical Na-K-2Cl cotransporter (NKCC2) and Cl channels, in the basolateral

membrane, move intracellular Cl^- down its chemical gradient into the interstitial fluid. K^+ reabsorption is also mediated here, primarily through potassium chloride (KCl) cotransport, allowing it to diffuse down its concentration gradient, being this diffusion dependent on systemic K^+ status. Furthermore, K^+ diffuses through apical K^+ channels known as outer medullary potassium channels, allowing transport of Na^+ and Cl^- on NKCC2, independently of the luminal K^+ levels. Cl^- absorption and K^+ secretion causes a positive lumen charge gradient, granting lumen-to-interstitium diffusion of Ca^{2+}, Mg^{2+}, Na^+ and K^+ through cation selective paracellular channels formed by claudin proteins in the thigh junctions (Verlander, 2020).

In the distal convoluted tubule Na^+ and Cl^- uptake is mediated by NaCl cotransporters, driven by basolateral Na-K-ATPase. Meanwhile, K^+ and Cl^- reabsorption works the same way as it does in the thick ascending limb, however, Ca^{2+} and Mg^{2+} uptake occurs trough transcellular route, mediated by specific transport proteins. By the time the filtered tubule fluid passes through here, more than 90% of salts have been reabsorbed. K^+ regulation is one of the most important roles of this portion of the tubules. After basolateral Na-K-ATPase pumps K^+ into the cells, it is sent to the interstitium by the basolateral potassium channel, which is also responsible for sensing changes in extracellular K^+ levels and regulating the signalling cascade that modifies the NaCl cotransporter phosphorylation, altering its transport activity. However, these effects on the cotransporter will affect not only K^+, but also Na^+ excretion. For example, increased dietary potassium will increase apical renal outer medullary potassium channels activity, enabling an increased K^+ secretion on the distal convoluted tubules and increasing aldosterone plasma levels, leading to increased Na^+ delivery to the collecting ducts. The result is an increase in Na^+ excretion by NaCl cotransporter inhibition and K^+ secretion stimulation. Low K^+ diets have the reverse effect (Verlander, 2020).

3.3.3. Collecting ducts

After the distal convoluted tubule there is a connecting segment between the nephrons and the collecting ducts. The initial portion of the collecting ducts transverse the cortex and medulla, ending in the papillary tip, where the tubule fluid discharges into the renal pelvis, where it is called urine. In the collecting ducts there are two types of cells: the intercalated cells, which has intracytoplasmic vesicles, mitochondria and a complex apical surface; and the principal cells, which represents most cells in this portion of the tubules, with less vesicles, mitochondria and a smooth apical surface, but with more basolateral membrane infoldings (Verlander, 2020).

Na^+ reabsorption is primarily a function of principal cells, driven by Na-K-ATPase pump like in the other tubule segments, actively transporting it into the interstitial fluid, which creates an electrochemical gradient favouring Na^+ uptake through apical epithelial channels and drives Cl^- absorption over the paracellular pathway through channels in the tight junctions formed by claudins. Type B intercalated cells, a subpopulation of intercalated cells, also contribute to NaCl reabsorption via apical Cl^-/HCO_3^- exchanger, pendrin and basolateral Cl channels (Verlander, 2020).

Net K^+ excretion is also modulated by the convoluted tubules, through basolateral Na-K-ATPase pumps located in principal cells, which send K^+ straight into the cell, raising intracellular levels above that of interstitial and tubule fluid, which in turn causes potassium to leave the cell through K^+ channels located in both apical and basolateral plasma membranes. Physiologically, this happens due to higher permeability of apical K^+ channels than basolateral channels and due to the lumen negative electrical potential, which benefits K^+ secretion. Regarding K^+ reabsorption, it is done in exchange of H^+, by apical hydrogen-potassium-adenosine triphosphatase (H-K-ATPase) isoforms like the ones in the gastric parietal cells. The reabsorption process will depend on systemic levels of K^+, which means that when dietary K^+ is restricted, there will be an inhibition of the secretion process, favouring K^+ exit through basolateral potassium channels (Verlander, 2020).

3.4. Hormonal control of solute reabsorption and secretion

Some specific homeostatic responses that regulate the rate of reabsorption of solutes are done by several hormones, including angiotensin II, aldosterone, antidiuretic hormone (ADH), endothelin-1, ANP, PTH, 1,25-dihydroxycholecalciferol (calcitriol), and calcitonin (Verlander, 2020).

As previously mentioned, angiotensin II is a modulator of GFR increasing blood pressure through the enhancement of sodium reabsorption in the renal tubules and collecting ducts. These segments have AT1 receptors that, when activated, increase Na^+ reabsorption, by stimulating all the different Na^+ exchange mechanisms present in the affected tubule segment (Verlander, 2020).

Aldosterone acts on the collecting ducts, activating the mineralocorticoid receptor located in the principal cells and connecting segment, and stimulates the Na^+ reabsorption pathways, which in turn also enhances water reabsorption to increase fluid volume. Furthermore, aldosterone affects Na^+ transport inside the cell, increasing the number of Na channels, thus enhancing its reabsorption. In addition, this hormone has an important role regulating K^+, as it is stimulated by hyperkalaemia. It will increase basolateral K^+ entry into principal cells, stimulating Na-K-ATPase activity, which, together with the increased Na^+ uptake, allows for a favourable gradient to be formed for K^+ secretion through specific channels, resulting in enhanced urinary excretion (Verlander, 2020).

ADH, or vasopressin, is responsible for increasing salt transport in case of hypotension or dehydration, acting mostly on the thick ascending limb and collecting ducts, by promoting the activity of NKCC2 and epithelial Na channels, respectively. It is notable that an increased sodium uptake will also increase water reabsorption because it contributes to interstitial osmolarity, allowing for more water from the diluted tubule fluid to be reabsorbed (Verlander, 2020). NO and endothelin-1 on the other hand increase Na^+ and water excretion. They work by inhibiting the sodium uptake mechanisms of different renal tubules, allowing for systemic regulation of blood pressure and extracellular fluid volume (Verlander, 2020).

ANP directly and indirectly inhibits Na^+ transport, by inhibiting renin and aldosterone release, limiting Na^+ reabsorption and increasing renal Na^+ excretion through suppression of Na-K-ATPase and Na channels activity, in the collecting ducts, and NKCC2, in the thick ascending limb (Verlander, 2020).

When it comes to phosphate reabsorption, many factors are involved, and it is usually reabsorbed coupled with Na^+. PTH inhibits phosphate transport and cotransporter expression by activating protein kinase A and protein kinase C (Lederer, 2014), but also increases inorganic phosphate (Pi) reabsorption from bone (Brito Galvao *et al.*, 2013). Calcitriol, the active form of vitamin D, also increases phosphate reabsorption from bone and intestinal absorption (Brito Galvao *et al.*, 2013). Calcium ions are also mostly passively reabsorbed in the proximal tubule, however, due to PTH stimulation, active transcellular transport of this ion is possible further down the renal tubules (Brito Galvao *et al.*, 2013). K^+ and Ca^+ are mostly reabsorbed through the paracellular pathway (Verlander, 2020).

Calcitonin, a hormone secreted by the thyroid gland, also regulates Ca^{2+} by reducing osteoclast-mediated bone resorption, increasing its deposit in bone. Furthermore, it enhances calcium excretion by enhancing its absorption in different portions of the renal tubules, despite the mechanism not being fully understood (Verlander, 2020).

Other regulators of both calcium and phosphate, are Klotho, which is an obligate co-receptor required for fibroblast growth factor 23 (FGF-23), increasing Ca^{2+} absorption by stabilizing membrane transporters and reducing Pi reabsorption by decreasing the expression of its cotransporter. FGF-23 is a bone-derived phosphaturic hormone, whose main function is to maintain Pi homeostasis by decreasing Pi reabsorption, thus increasing phosphaturia and inhibiting Pi gastrointestinal absorption when there are reduced levels of calcitriol. FGF-23 and calcitriol create a feedback loop (Figure 5), which means that when high levels of calcitriol and Pi are present, the production of FGF-23 is stimulated, decreasing production of calcitriol, which in turn causes a decrease in FGF-23 production. This loop then affects Pi levels. FGF-23 also inhibits the release of PTH from the parathyroid gland, connecting to the Klotho receptors present here (Brito Galvao *et al.*, 2013).

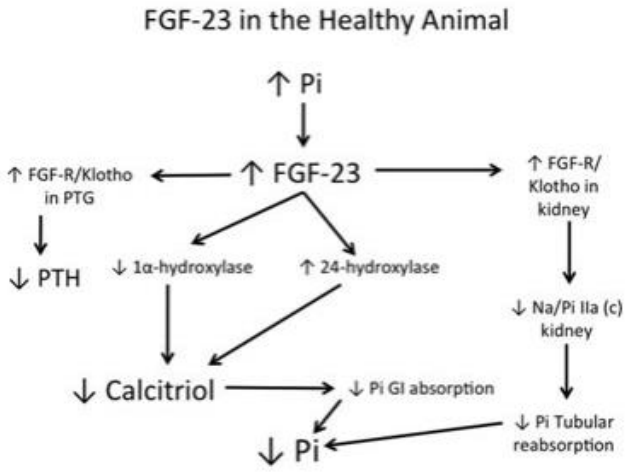


Figure 5 - FGF-23's action over Pi levels and Calcitriol. Source: (Brito Galvao et al., 2013)

3.5. Water balance

As mentioned before, the kidney can produce concentrated or diluted urine as needed, due the generation of a hypertonic interstitium or due to the thick ascending limb and interstitial fluid, respectively. Variability in water permeability on the collecting ducts when responding to ADH also causes these fluctuations in urine concentration (Verlander, 2020).

Most of the water that is filtered in the glomerulus is reabsorbed in the proximal tubule by both active and passive mechanisms. The Na-K-ATPase pump removes solutes from the tubule fluid, which in turn allows the creation of a small gradient that favours water uptake to the cells and intercellular spaces. The apical brush border and basolateral infolding increase the surface area that contacts with water, making it highly permeable due to numerous H_2O channels that extend throughout the tubule. Besides these, in the tight junctions there are channels formed by claudin-2 that facilitate paracellular water transport (Verlander, 2020).

The thick ascending limb, despite being impermeable to water, contributes to the hypertonicity of the interstitium, allowing water than to be reabsorbed on the descending limb, creating a countercurrent mechanism (Figure 6) (Verlander, 2020).

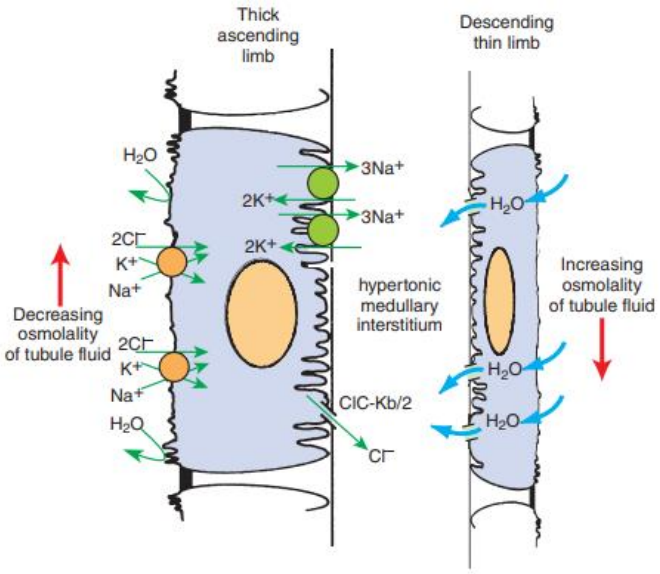


Figure 6 - Countercurrent mechanism. In green are the Na-K-ATPase and in orange the NKCC2 transporters. Source: (Verlander, 2020)

The vessels also participate in the countercurrent mechanism, due to its permeability to salts, urea, and water. The *vasa recta* favour the movement of water into the capillary lumen, allowing for an equilibrium in luminal NaCl and urea levels with the interstitial concentrations. Therefore, as the vessels descend into the inner medulla, the plasma osmolality and urea concentrations increase in the *vasa recta*, near the end of the loop of Henle, and then fall as the vessel ascend out of the medulla. The medulla has a progressively higher osmolality in the tubule fluid and interstitial fluid the deeper it goes due to the diffusion of salts and urea that happen in this portion of the tubules (Verlander, 2020).

3.6. Urea recycling

The inner medullary collecting duct is important for the reabsorption of urea as most of the tubule is impermeable to it. This segment of the collecting ducts possesses specific urea transporters, allowing the reabsorption. The thin limb of the loop of Henle are also permeable to urea, receiving it from the *vasa recta*. This means that urea travels from blood, diffusing out of the fenestrated capillaries into the interstitial space and then entering the thin limb of the loop of Henle down its concentration gradient. After this, urea recycling only happens in the inner medullary collecting ducts, where it returns to the interstitium. ADH enhances urea reabsorption, increasing interstitial osmotic pressure, hence increasing water reabsorption (Verlander, 2020).

3.7. Acid-base balance

The kidneys, the lungs and intracellular and extracellular buffers work together to maintain the blood normal pH. The kidney does this by generating and maintain normal blood levels of bicarbonate and excreting hydrogen ions, thanks to the action of carbonic anhydrases, H^+

transporters in the renal tubules that exchange this ion for solutes, buffers that bind to protons, minimizing H^+ concentrations in the tubule fluid and the renal ammonia metabolism and secretion. The majority of filtered bicarbonate is reabsorbed; however, it needs to be first combined with H^+ to form carbonic acid, which in turn is converted to CO_2 and H_2O. CO_2 then will cross the plasma membrane into the cell, where it will react with carbonic anhydrase to form H^+ and HCO_3^- (Verlander, 2020).

The kidney secretes H^+, primarily through the proximal tubule, however the collecting duct will be the final controller of net acid excretion and urine pH. The main route, in the proximal tubule and thick ascending limb, for renal excretion of hydrogen ions is the Na^+/H^+ exchanger. Filtered phosphate will also interact with H^+, making so that the free form of phosphate, hydrogen phosphate (HPO_4^{2-}), is titrated to form phosphoric acid ($H_2PO_4^-$), which is retained in the tubule fluid and eliminated, working as a buffer for urinary titratable acidity. Other luminal buffers are creatinine and citrate (Choi, 2008; Verlander, 2020).

Luminal ammonia is the other major buffer, that unlike phosphate and bicarbonate, almost exclusively arises from proximal tubule production and secretion rather than glomerular filtration, because the amino acid glutamine is metabolized, producing not only ammonia (NH_3) and H^+, but also bicarbonate. This reaction is stimulated in case of metabolic acidosis, as a renal response mechanism to an increase in the acid load. However, all NH_3 is going to be essentially presented in ammonium (NH_4^+) form, due to the elevated pKa of NH_3. This NH_4^+ will enter the renal tubules through carrier mediated transport, reabsorbed in exchange for K^+ on the apical NKCC2. NH_4^+ will be formed by binding NH_3 with H^+, lowering both of their concentration, which ultimately contributes to raising tubule fluid pH and maintains of a favourable NH_3 gradient (Verlander, 2020).

4. Chronic Kidney Disease

Chronic kidney disease (CKD) is characterized as a progressive loss of kidney function, in one or both kidneys, during an extended period (usually over three months), being one of the most frequently encountered disorders in senior and geriatric cats. It may result from any condition that causes continuous damage to the kidneys, being usually described as a multifactorial disorder. The severity of the illness is denoted by the IRIS staging system according to the degree of azotaemia, ranging from stage I, which represents non-azotaemic disease, to stage IV, severe renal azotaemia (Reynolds & Lefebvre, 2013; *IRIS Kidney - Guidelines - IRIS Staging of CKD*, 2023). Substaging of CKD depends on the presence of either proteinuria or hypertension (*IRIS Kidney - Guidelines - IRIS Staging of CKD*, 2023). These guidelines allow not only for diagnosis of CKD in cats, but also guide veterinarians in establishing a treatment plan to the patients' prognosis (Hall *et al.*, 2019).

Because CKD is uncurable, treatment is overall symptomatic, based on a conservative approach in order to try preventing further progression of the disease and maintaining the patients' quality of life (Sparkes *et al.*, 2016).

4.1. Epidemiology and Risk factors

CKD in cats is a prolonged process characterized by irreversible loss of kidney function and with an increasing prevalence for the past decades, affecting up to 80% of senior and geriatric cats (Marino *et al.*, 2014; Chen *et al.*, 2020).

A study was conducted in the United Kingdom on mortality rates associated with renal disease in which around 13.6% of deaths were caused by CKD in cats older than five years (Brown *et al.*, 2016). The mean survival time for stable cats with symptomatic, or stage II, CKD was around 1151 days, becoming smaller has the disease progresses, although it is also admitted that overall survival was subjected to interindividual variability, modalities of therapy, interventions, and resources available (Jepson, 2016).

It is also estimated that clinical signs of the disease happen when azotaemia is present, which happens when 75% of the kidney function is compromised, however, the few remaining healthy glomeruli can occult this loss in kidney function for a determined amount of time, making it harder diagnose the disorder in its earlier stages (Böswald, Kienzle & Dobenecker, 2018; Conroy *et al.*, 2019). Considering that the nephron damage is irreversible it is essential to identify, manage and/or prevent risk factors that can contribute to the progression of CKD in cats. Some examples of these are age, breed, vaccinations, hypertension, proteinuria, hyperphosphatemia, and acute kidney injury (AKI), to name a few (Chen *et al.*, 2020).

There is also some evidence that link feline immunodeficiency virus (FiV) and feline leukaemia virus (FeLV) infections with CKD, thanks to the formation of immunocomplexes (Rossi *et al.*, 2019). Furthermore, there is a study suggesting that cat vaccines, because of the use of Crandal-Rees feline kidney (CRFK) to grow these pathogens, there is a formation of cell lysates, which in turn can lead to the development of antibodies against the kidney cells leading to nephropathies (White, Malik & Norris, 2011). Other infections, like urinary tract infections (UTI), are also considered risk factors for developing CKD, especially if it becomes a chronic problem, as they are link to pyelonephritis and renal fibrosis in cats. Conversely, CKD can also lead to more frequent UTI thanks to urodynamic alterations (Reynolds & Lefebvre, 2013).

Nephrotoxic drugs, like nonsteroidal anti-inflammatory drugs (NSAID), aminoglycosides or antineoplastic agents can cause acute kidney injury that if left untreated may evolve to CKD (Reynolds & Lefebvre, 2013). NSAID inhibit cyclooxygenase enzymes within the kidney, decreasing the release of vasodilatory agents responsible for maintaining normal renal blood flow (Jepson, 2016).

An inappropriate diet, high in protein and phosphorus and depleted in potassium, exclusively fed during several years, was shown to induce CKD in healthy cats. These diets, besides leading to hypokalaemia, can also lead to azotaemia (Finch, Syme & Elliott, 2016).

Ageing is considered a risk factor thanks to its relationship with the disease and its progression because mortality rates and severity of kidney injury increase with age. Some effects of ageing that could be related with CKD are mitochondrial dysfunction, heightened intrarenal inflammatory response, and increased cellular senescence. Ageing, together with

hyperphosphatemia, anaemia, hypertension, and proteinuria are viewed as promoters of renal fibrosis, which is thought to be the main lesion seen in CKD, primarily in the tubulointerstitial space (Brown *et al.*, 2016).

4.2. Aetiology

Besides ageing and renal hypoxia, the initiators of CKD are still unknown, being these the only factors admitted as primary causes, as they are constantly identified in cats with CKD (White, Malik & Norris, 2011). It is considerate to be an multifactorial disease, with both congenital and acquired possible factors (Reynolds & Lefebvre, 2013; Thornton, 2017).

Regarding congenital diseases, these are usually associated with genetics, meaning that some cat breeds will be more predisposed to certain diseases which might lead to CKD, such as: autosomal dominant polycystic kidney disease, one of the most seen conditions that affect Persian and Persian-cross cats; renal amyloidosis, commonly cognate to Siamese, Oriental and Abyssinian cats; glomerular disease; and juvenile renal dysplasia in Abyssinian (Reynolds & Lefebvre, 2013).

Acquired diseases that have been identified, or suspected, in many cases of feline CKD include conditions that affect the urinary tract directly or systemic disorders that are related to kidney damage. For instance, the presence of uroliths in the upper urinary tract is diagnosed in around 15% to 29% of CKD cats (Reynolds & Lefebvre, 2013). Moreover, urethral obstructions are also one of the most common urinary tract diseases in cats, that may lead to acute azotaemia and, if they are a recurrent problem, to gradual loss in kidney function (Reynolds & Lefebvre, 2013; Chen *et al.*, 2020). Cats with renal lymphoma may also have CKD associated, being that 60% of cats with this disease present azotaemia (Reynolds & Lefebvre, 2013).

Some systemic diseases, like hyperthyroidism or infections, may also be potential triggers for CKD. Hyperthyroidism is commonly seen together with CKD, being usually identified in elder cats. Actually, some studies show that 15% to 51% of cats with hyperthyroidism present underlying CKD, which it is implicated in the progression of the disease due to the effects the thyroid hormone has on the kidneys, like hyperfiltration, altered renal haemodynamic, and increased proteinuria. Both are also known for supressing each other's symptoms, making it harder to achieve diagnosis (Jepson, 2016; Geddes & Aguiar, 2022; Yu *et al*., 2022).

4.3. Pathophysiology

CKD occurs due to an adaptive mechanism that, at first is advantageous to the patient by "masking" the loss of nephrons, but eventually becomes detrimental to the kidney's function. When the kidney suffers an initial lesion, fibrosis will take place in focal areas of inflammation and there will be activation of mesenchymal cells, in order to fill the damaged space. However, this may interfere with the full recovery of the kidney, not allowing it to repair its functional parts. Moreover, if an excessive fibrogenic response takes place, together with an expansion of the

extracellular matrix, there will be destruction of the normal kidney tissue, leading to CKD (Reynolds & Lefebvre, 2013; Jepson, 2016). CKD is also associated, due to some alterations in the glomeruli, to increased intraglomerular capillary pressure and other changes that impair glomerular filtration, resulting in increased losses of albumin and other proteins, which in turn contributes to the progression of the disease by promoting tubular inflammation and fibrosis (Sparkes *et al.*, 2016). There is some evidence of altered antioxidant status in cats with CKD, which can support the hypothesis that hypoxia plays a role in CKD progression (Jepson, 2016).

Transglutaminase 2 (TG2) plays a role in the extracellular matrix (ECM) formation by creating bonds between matrix proteins, displaying a transamidase activity, stabilizing the formed matrix, helping in cell differentiation or cell adhesion and promoting ECM deposition, which will also increase its resistance to breakdown via proteolytic enzymes. In addition to this, TG2 will also be involved in cross-linking the transforming growth factor–β1 (TGF-β1) with the ECM contributing to tissue fibrosis by TGF-β1 activation, as well as stimulating the pro-inflammatory signalling. Therefore, there is a strong association between TG2 expression and renal fibrosis in CKD. Besides these changes, extracellular TG2 is also correlated with biochemical markers of kidney disease, like creatinine, urea and phosphate (Lawson *et al.*, 2015; Prat-Duran *et al.*, 2021; Kongtasai *et al.*, 2022).

4.3.1. Kidney morphologic changes

Has mentioned before, a maladaptation on the kidney's response to lesions will lead to morphological changes in its structure. Macroscopically, the kidney will appear smaller in size and present surface pitting. Moreover, marked dilation of the afferent arterioles is also visible and at palpation they have harder consistency than normal (Brown *et al.*, 2016).

Histologically, cats will have primary lesions found in the interstitial compartment, with only mild, presumed secondary sclerotic lesions in the glomeruli, in contrast with the majority of the domestic species with CKD, where primary glomerular disease is found. These lesions are multifocal or segmental, and present interstitial mononuclear cell inflammation, tubular degeneration and atrophy, interstitial fibrosis, mineralization of Bowman's capsule and tubular basement membranes, interstitial lipid, and glomerulosclerosis. As the disease progresses, so do the lesions, becoming more severe. Interstitial fibrosis, being the most common histologic finding, will be correlated with the severity of azotaemia (Brown *et al.*, 2016). The presence of lipids in the interstitial space, fairly common finding in cats with stage II to IV CKD, may occur due to tubular ischemia and rupture, which releases intraepithelial lipids accompanied by granulomatous inflammation (Brown *et al.*, 2016).

As the kidney starts to lose its glomeruli, it will compensate for that loss by increasing the working glomeruli size, with also some degree of mesangial matrix expansion (glomerulosclerosis). These hypertrophied glomeruli will start to receive and filtrate more blood (hyperperfusion), causing podocyte damage and loss, allowing for excessive protein filtration (Taylor & Sparkes, 2013). Generalized glomerulosclerosis seems to be normal in aged cats, but

it is also a pathologic change that increases in severity throughout the progression of the disease, causing collapse of the capillary tuft, thickening of the GBM and fibrosis of the urinary space, which is consistent with ischemic alterations rather than the progression of focal segmental glomerulosclerosis (Brown *et al.*, 2016).

4.3.2. Hyperphosphatemia

CKD is the most common cause of hyperphosphatemia in cats, and it happens due to renal excretion impairment, which allows the retention of excess phosphate. This can promote renal secondary hyperparathyroidism, which is linked with increased mortality in all species due to its contribution to uraemia and disease progression, mineralization of tissues, and progression of CKD by promoting inflammation and fibrosis (Polzin, 2011; Jepson, 2016; Sparkes *et al.*, 2016).

In early CKD, increased levels of PTH can maintain phosphorus levels within the reference range, allowing normalization of serum Pi levels at the cost of hyperparathyroidism. However, when GFR drops to 20% or less, this adaptive response is no longer capable of maintaining Pi levels, leading to hyperphosphatemia (Kidder & Chew, 2009). This retention inhibits 1α-hydroxylase, an enzyme responsible for activating vitamin D3, also known as calcitriol, which is important for calcium and phosphorus intestinal absorption and for inhibiting PTH synthesis. Pi has been shown to stimulate PTH synthesis independently of changes in calcium and calcitriol (Figure 7) (Kidder & Chew, 2009).

Hyperphosphatemia and renal secondary hyperparathyroidism are common findings in cats with IRIS stage III and IV and can also be documented in IRIS stage II (Kidder & Chew, 2009).

Phosphate may also promote cellular apoptosis, cellular senescence and oxidative stress, which makes the patients predisposed to having necrosis and calcification of the convoluted tubules and formation of excessive extracellular matrix, due to calcium phosphate precipitation into the tissues. Furthermore, extracellular Pi is associated with the stimulation of the RAAS and it increases the production of profibrotic mediators (Jepson, 2016).

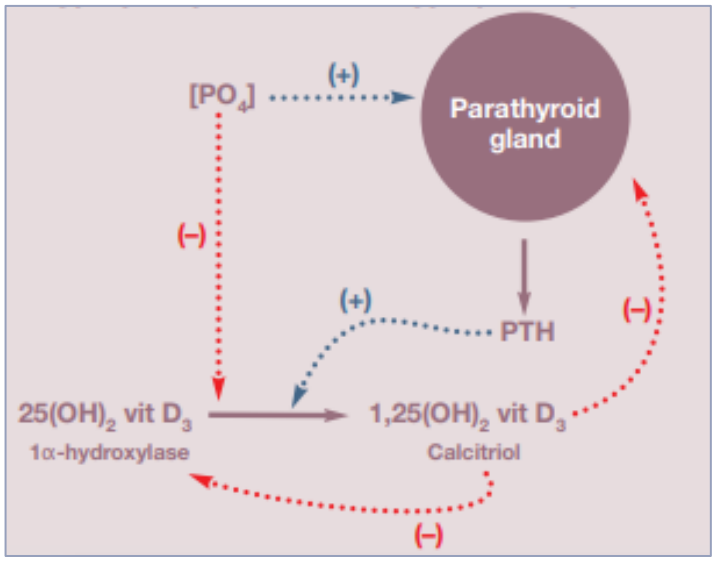


Figure 7 - The relation between hyperphosphatemia and hyperparathyroidism. (+) stimulates; (-) inhibits. Source: (Kidder & Chew, 2009)

4.3.3. Proteinuria

The presence of protein in urine, such as albumin, immunoglobulin G and transferrin, upregulate the production of vasoactive, pro-inflammatory and profibrotic factors, which is related to tubular atrophy, proximal tubule cell apoptosis and glomerular hypertrophy, therefore being used has a marker of tubular dysfunction in cats (Jepson, 2016; Reynolds & Lefebvre, 2013). It is also believed that non-esterified fatty acids and long-chain acyl-coenzyme A bound to albumin may accumulate in the proximal tubule and promote apoptosis. Non-esterified fatty acids travel through the blood bound to albumin and, as they reach the kidney, they dissociate (due to changes in pH or interactions with other transport proteins). Once dissociated, these interact with tubular epithelial cells, leading to apoptosis (Jepson, 2016).

Comparatively with other species, the degree of proteinuria is typically low in cats, due to their primary histopathologic lesion being tubulointerstitial, whereas dogs, for example, have more marked proteinuria in CKD cases. Nevertheless, proteinuria in cats has been associated with the development of azotaemia and reduced survival in cats with both CKD and hypertension (Reynolds & Lefebvre, 2013; Jepson, 2016)

Some biomarkers used to detect glomerular or tubular damage are proteins with intermediate and high molecular weight or low molecular weight, respectively. Usually, low molecular weight proteins are undetectable in urine, although, as tubulointerstitial damage starts to appear in feline CKD, their reabsorption is affected. Moreover, tubular cells secrete these proteins in response to tubular damage, increasing even more their concentration in the urine (Kongtasai *et al.*, 2022).

4.3.4. Renal secondary hyperparathyroidism

With the progression of CKD, fewer healthy proximal tubule cells will release 1α-hydroxylase, responsible for converting calcidiol into calcitriol, leading to hypocalcaemia due to

decreased intestinal calcium absorption, which in turn stimulates PTH secretion. On the other hand, as long as there are enough healthy tubular cells to produce the 1α-hydroxylase enzyme the increase in this hormone's secretion can restore ionized calcium and calcitriol serum levels in early CKD (Figure 8) (Brito Galvao *et al.*, 2013).

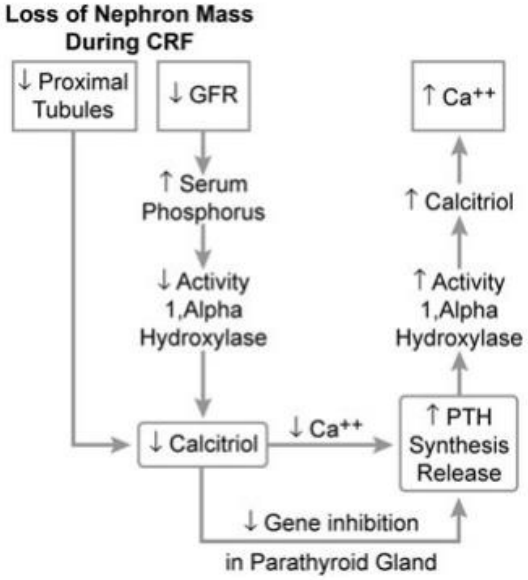


Figure 8 - Development of renal secondary hyperparathyroidism due to decreases in calcitriol and calcium in early CKD. Source: (Brito Galvao et al., 2013)

It is also known that FGF-23 secretion is increased in CKD, however Klotho expression in the parathyroid gland is decreased in these patients, downregulating the Klotho/FGF complex. Therefore, this rise in FGF-23 levels will not decrease PTH secretion (Brito Galvao *et al.*, 2013). In the earlier stages of CKD (stages I and II), with the increase of phosphate serum concentrations due to decreases in healthy nephrons and GFR, FGF-23 is stimulated, reducing phosphate serum levels by increasing the urinary excretion (phosphaturia) and indirectly inhibiting Pi intestinal absorption, lowering calcitriol synthesis. These interactions allow the maintenance of normal serum phosphate in these stages of CKD (Polzin, 2011) (Figure 9).

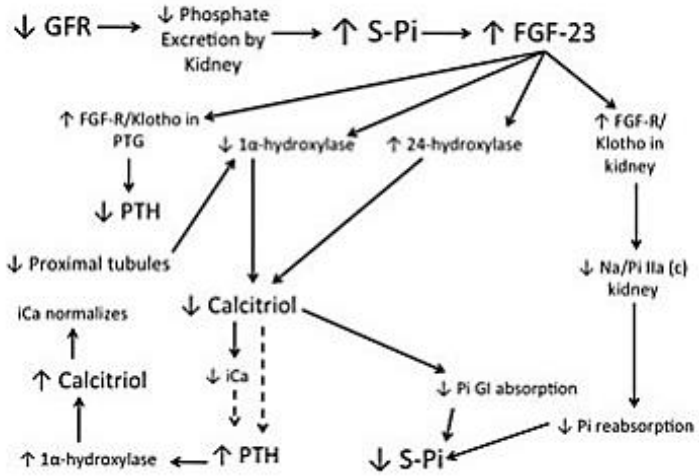


Figure 9 - The role of FGF-23 in early CKD. Source: (Brito Galvao et al., 2013)

However, as the disease progresses and GFR further declines, serum increases of phosphate become more severe, further stimulating FGF-23 release. Like mentioned before, with the reduction in healthy proximal tubule cells, less 1α-hydroxylase enzyme is produced, lowering calcitriol production and consequently ionized calcium concentration. There will then be a spike in PTH in order to regulate calcitriol and calcium, but it will also contribute to increases in phosphate levels. Thus, in order to maintain calcium concentrations, elevated PTH and serum phosphate levels may be observed. In some early CKD cases there may be hyperparathyroidism regardless of serum phosphate levels (Brito Galvao *et al.*, 2013; Broek, van den *et al.*, 2022).

When most of the kidney function is lost, there is an absolute decrease in GFR, leading to significantly higher concentrations of serum phosphate, which in turn leads to higher FGF-23 levels. Calcitriol and ionized calcium levels will also be decreased due to the accentuated loss of proximal tubular cells, which in turn stimulate PTH production. However, in this stage, it can no longer upregulate calcitriol synthesis, meaning ionized calcium will remain low despite PTH increasing. Furthermore, with a decrease in total healthy renal tubules, klotho expression will also be affected, with serum klotho levels declining as CKD progresses (Lu & Hu, 2017), limiting FGF-23 action to excrete phosphate and reduce PTH synthesis. In addition, with the upregulation of 24-hydroxylase, there will also be an increase in calcitriol degradation, making it impossible to maintain calcium levels (Figure 10). Thus, in late CKD it is possible to find hypercalcaemia, followed by hypocalcaemia, hyperphosphatemia, and hyperparathyroidism (Polzin, 2011; Brito Galvao *et al.*, 2013; Broek, van den *et al.*, 2022).

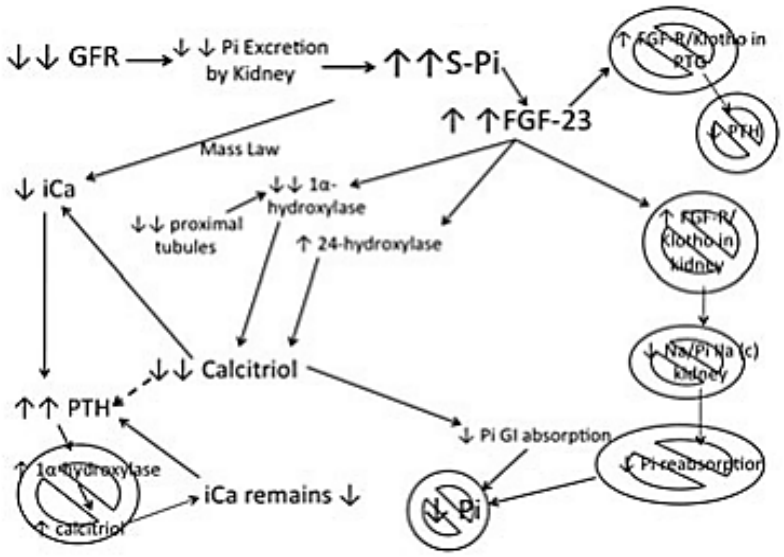


Figure 10 - The role of FGF-23 in late CKD. Source: (Brito Galvao et al., 2013)

Therefore, renal secondary hyperparathyroidism can be classified as an maladaptive process that develops in order to recover calcitriol and calcium levels, and to reduce phosphate levels (Reynolds & Lefebvre, 2013).

Because of the associations between FGF-23, calcitriol and PTH with kidney damage they can be related with azotaemia and uraemia in cats with CKD. For instance, the more PTH values rise, the more severe the azotaemia. In addition, the higher the FGF-23 values are, the more likely azotaemia and hyperphosphatemia will be present (Brito Galvao *et al.*, 2013; Finch *et al.*, 2013). SDMA concentrations are also related with higher FGF-23, which is to be expected, since SDMA and creatinine are indirect biomarkers of GFR and FGF-23 increases with its reduction (Korman & White, 2013; Kongtasai *et al.*, 2022).

4.3.5. Renin-angiotensin-aldosterone system

The RAAS is a major contributor to progressive renal injury. It works as a compensatory mechanism to increase GFR via constriction of the afferent arteriole, however, this response becomes maladaptive, resulting in proteinuria and in the development of hypertension. This, together with dehydration and activation of the sympathetic nervous system, compromise the renal microcirculation. The presence of other vasoconstrictors, like thromboxane A2 or endothelin could augment this constriction and lead to segmental tubular hypoxia, which may be relevant to the progression of CKD in cats, being that dehydration and stress are common findings in aged cats, particularly those with the disease. Endothelial dysfunction is also believed to contribute to this, as the endothelium of the kidney's blood vessels becomes impaired of releasing vasodilator substances, such as NO, and having no way of counterbalancing the vasoconstrictors (Brito Galvao *et al.*, 2013; Brown *et al.*, 2016; Lawson & Jepson, 2021).

Angiotensin II stimulates the epithelial-to-mesenchymal transition, the transforming growth factor and the connective tissue growth factor β, therefore, functioning as a major fibrogenic cytokine, besides also contributing to glomerular hypertension and hyperfiltration. Thus, it is believed that it upregulates the uremic progression and not the high blood pressure *per se* (Brown *et al.*, 2016; Jepson, 2016). Hypertension itself is not considered a risk factor for CKD progression in cats, but a risk factor for the development of proteinuria instead, because glomerular capillary hypertension will increase the amount of protein in the filtrate, which is believed to be intrinsically toxic for renal tubule cells leading to inflammation and further nephron loss (Lawson & Jepson, 2021).

Calcitriol can supress renin biosynthesis and can supress the induction of angiotensinogen by blocking the nuclear factor pathway, slowing RAAS, however, as disease progresses and calcitriol levels start to drop, it no longer can reduce the systems' activation (Brito Galvao *et al.*, 2013).

4.3.6. Hypoxia

In early CKD, the changes in glomerular structure and development of glomerulosclerosis may alter the blood supply of the kidney. In order to compensate for nephron loss and drops in GFR, hemodynamic adaptations, driven by the activation of the RAAS and angiotensin II, cause vasoconstriction of the afferent arterioles and, in turn, enhance glomerular capillary pressure and filtration. However, these changes may bring consequences to the blood supply and oxygen delivery, resulting in hypoxia (Jepson, 2016).

Hypoxia stimulates tubular epithelial cells to undergo epithelial-mesenchymal transition (EMT), which plays an important role in the development of renal fibrosis (Seccia *et al.*, 2019) and activates fibroblasts to increase the formation of extracellular matrix. Some cells, when exposed to lower levels of oxygen, develop mitochondrial derangements that result in cellular apoptosis. Furthermore, another consequence of this is the decrease in the number of available fibroblasts, as there is loss in renal parenchyma, reducing the production of endogenous erythropoietin that later in the disease may cause anaemia contributing to reduced oxygen delivery and hypoxia (Figure 11) (Jepson, 2016).

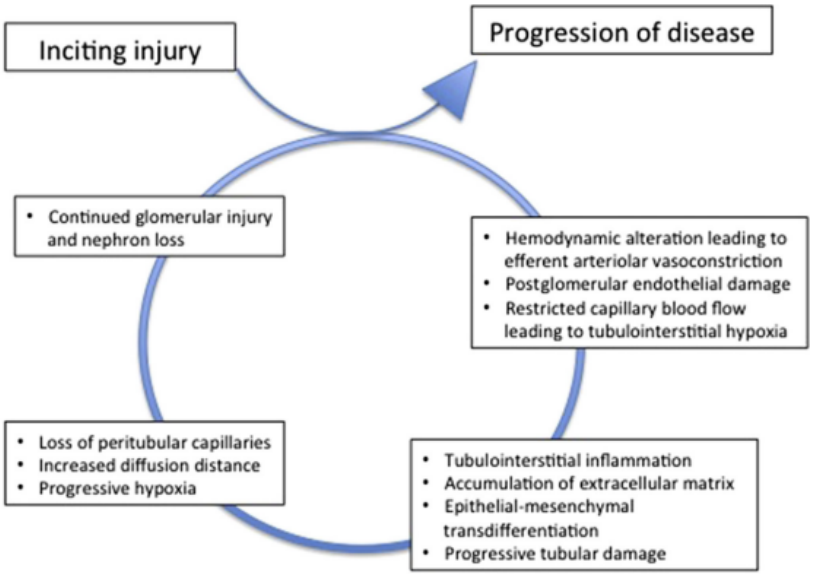


Figure 11 – The mechanism behind the development of CKD-induced hypoxia. Source: (Jepson, 2016)

4.3.7. Oxidative Stress

Renal oxidative stress can occur when there are imbalances between the production of reactive oxygen species (ROS), highly reactive molecules that damage DNA, lipids, proteins and carbohydrates. The hyperfiltration and hyperfunctioning of the remaining nephrons lead to increases in the production of these reactive species, resulting in cellular apoptosis and necrosis, stimulating inflammation and fibrosis, and reducing the availability of antioxidant defence mechanisms. Therefore, cats with CKD have reduced antioxidant capacity and a higher reduced/oxidized glutathione (GSH:GSSG) ratios, a system that maintains the concentrations of ROS at physiological levels, because of the hyperfunctional adaptability of the remaining nephrons, which leads to a dramatic increase in cellular oxidative phosphorylation (Brown, 2008; Jepson, 2016).

Besides this, changes caused by age, RAAS activation and chronic systemic inflammation or chronic inflammation of the kidney may promote the formation of ROS. For example, interstitial fibrosis and areas of renal ischemia cause mitochondrial dysfunction and increased oxygen leaking, contributing to further ROS generation. Anaemia, a frequent finding in patients with CKD, exacerbates even more this problem, because hypoxia enhances ROS production and erythrocytes have an antioxidant function in the kidney (Brown, 2008).

Hypertension creates a positive feedback loop with oxidative stress, being believed that the ROS can stimulate an elevation in systemic blood pressure, which in turn can promote GFR, thus contributing to the hyperfunctioning of the nephron in CKD (Brown, 2008).

4.3.8. Anaemia

Anaemia is commonly found in cats with CKD, being present in 30% to 65% of affected patients, usually identified as non-regenerative (normochromic, normocytic and hypoproliferative) (Korman & White, 2013; Brown *et al.*, 2016). It results from insufficient renal erythropoietin (EPO)

production, predisposing the kidney to hypoxia. This anaemia could be exacerbated from gastrointestinal haemorrhage, malnutrition and reduced red blood cell lifespan, which can also lead to iron deficiency, limiting the body's capacity to produce haemoglobin, and therefore exacerbate the anaemia. Furthermore, the total iron binding capacity of the kidney is limited in cats with CKD, due to the chronic inflammatory process that takes place. Moreover, uraemia affect red cell lifespan and can also be considerate responsible for gastrointestinal haemorrhage and malnutrition due to sickness. (Korman & White, 2013; Reynolds & Lefebvre, 2013; Gest, Langston & Eatroff, 2015; Brown *et al.*, 2016; Quimby, 2016).

Hepcidin, an acute-phase protein that regulates iron homeostasis, is upregulated in inflammation, and leads to sequestration of iron, therefore negatively affecting iron absorption in the intestines. For that reason it is thought to be a central mediator of anaemia in chronic disease, like CKD (Quimby, 2016).

Anaemia is identified as a risk factor for CKD progression and affects both quality of life and survivability. In order to fight anaemia, the body increases the release of norepinephrine, renin, angiotensin II and aldosterone, which can lead to hypertension. It may also cause heart problems, as it will try to compensate for the less available red blood cells (Quimby, 2016).

4.3.9. Azotaemia and Uraemia

Azotaemia is a condition defined by an elevation or build-up of nitrogenous products, like blood urea nitrogen (BUN), creatinine in the blood and other secondary waste in the body. It may have multiple origins, being classified as pre-renal, renal, or post-renal depending on said origin. Renal azotaemia is related with CKD and other kidney related diseases, and it confirms the presence of renal damage (Tyagi & Aeddula, 2023).

Uraemia is a syndrome resultant of kidney damage, characterized by fluid, electrolyte, hormonal and metabolic abnormalities. It develops most commonly in chronic and end-stage kidney disease but may also be found in AKI if loss of functional nephrons happens quickly. Clinical signs of urea are diverse, being the most common nausea, vomit, fatigue, anorexia, muscle cramps and altered mentation. Urea itself is considered toxic to some tissues and promotes anaemia, by reducing erythrocyte half-life, gastropathies like ulcerative haemorrhagic gastritis (most common uraemic gastropathy in cats), and other non-renal lesions (Ambrosio *et al.*, 2020; Zemaitis *et al.*, 2023).

It is also important to know that azotaemia may occur without uraemia or kidney disease (pre-renal azotaemia). In opposition, although uraemia does not develop without the presence of azotaemia, it can manifest itself without any kidney disease, for instance in uroabdomen cases (Polzin, 2017).

4.4. Diagnosis

CKD is diagnosed based on the identification of compatible clinical signs and presence of renal azotaemia. Although the diagnosis of late stage CKD is relatively straightforward, the

detection of early kidney disease is challenging, as patients may present little to no evidence of impaired renal function. Ideally early CKD would be detected by determining GFR directly, however its use in routine clinical practice is hampered due to the cost and difficulty in identifying early disease. Hence, more detailed and frequent health assessments should be carried out in senior cats, as they are more predisposed to develop CKD. These should ideally include full history and physical examination, routine urinalysis, routine blood works (biochemistry and haematology), blood pressure measurements and diagnostic imaging (Paepe & Daminet, 2013; Sparkes *et al.*, 2016). Other complementary means for diagnosis can be used, like histologic studies, as an attempt to recognize specific abnormalities in the kidneys structure (Polzin, 2011).

4.4.1. History and physical examination

Cats with CKD may appear in consult at any stage of the disease. Therefore, the case presentation will vary. Firstly, age and breed need to be identified, as senior cats are more predisposed, and some specific breeds may also show an increased risk of developing CKD secondary to a congenital disease. Furthermore, it is important for the veterinary to understand the evolution of the illness, whether the onset was acute or gradual, and to assess the progression of the clinical signs. Moreover, the owner should be enquired about the cat's diet, water intake, urine frequency, its (in)capacity to empty the bladder and if there is any previous history of obstruction. In addition, unspecific signs, such as vomiting, anorexia, halitosis may also be relevant to better understand the disease (Paepe & Daminet, 2013; Sparkes *et al.*, 2016).

When performing a physical exam, special attention should be given to body and muscle condition, body weight and its evolution, hydration status, presence of periodontal disease, colour and humidity of the mucous membranes, hair coat condition and to the kidneys' size, shape and consistency, as well as sensibility to palpation. Furthermore, pulse quality and blood pressure measurements should also be made, whenever possible (Paepe & Daminet, 2013; Polzin, 2017).

4.4.2. Clinical signs

As mentioned, clinical manifestations of CKD vary from patient to patient, depending on the stage of the disease and for how long the animal has been affected by it. Because CKD is a progressive disease, clinical signs become more evident as it advances, therefore stage I disease is hard to identify, having no, or only a few, symptoms, whereas stage IV disease is more easily identifiable, as it presents more severe systemic clinical signs (Paepe & Daminet, 2013; Reynolds & Lefebvre, 2013; Jepson, 2016; Geddes & Aguiar, 2022; Grelová *et al.*, 2022;).

One of the most consistent clinical signs in early CKD is the inability to concentrate urine, which results in polyuria and compensatory polydipsia. Other common, nonspecific symptoms include inappetence, weight loss, lethargy, halitosis, vomiting, and dehydration. Some animals may present periodontal disease, gingivitis, and oral ulceration, due to an ongoing uremic stomatitis and cardiovascular complications (Reynolds & Lefebvre, 2013; Geddes & Aguiar, 2022).

4.4.3. Laboratory exams

In order to obtain a more definitive diagnose of CKD, azotaemia and reduced urine specific gravity (<1.035) should be identified. When azotaemia is detected, it is necessary to identify its origin, and it can be done by ruling out pre-renal and post-renal azotaemia. The first can be discarded either by analysing the patient's urine specific gravity (USG) or through clinical trial (i.e., rehydration). The latter is excluded by the patient's history, if there are no recordings of any previous obstructions and/or incapacity to empty the bladder (Chen *et al.*, 2020; Groves, 2020).

4.4.3.1. Staging

The International Renal Interest Society (IRIS) developed a staging system based on blood creatinine and SDMA concentrations. Substaging is done according to blood pressure and degree of proteinuria. This facilitates the application of clinical practice guidelines for diagnosis, treatment and prognosis. The IRIS guidelines also include reference ranges for phosphate in cats, and the optimal target for every CKD stage (Groves, 2020; *IRIS Kidney - Guidelines - IRIS Staging of CKD*, 2023).

Urea and creatinine are used as biomarkers for renal function. However, these are insensitive markers for GFR, only rising when a large portion of functional nephrons has been lost and may be affected by other factors such as hydration, muscular mass, protein intake and other non-renal conditions. Creatinine is preferred over urea as it is completely filtered by the kidney at a constant rate, but its exponential relation with GFR means it will evolve slowly in early disease, whereas in late CKD it may suffer large sudden changes as GFR get more affected (Groves, 2020; *IRIS Kidney - Guidelines - IRIS Staging of CKD*, 2023).

SDMA is a more recent biomarker, more sensitive than creatinine for early renal disease whose levels are not affected by muscle wasting and can be detected before azotaemia is present. SDMA plasma concentrations correlate with GFR as it starts rising as soon as 25% to 40% of renal function is lost. It is suggestive of reduced renal function when patients present persistent values of SDMA over 14 mg/dL even without present azotaemia, which may be indicative of stage I disease (Groves, 2020; *IRIS Kidney - Guidelines - IRIS Staging of CKD*, 2023).

SDMA and creatinine should not be used to diagnose CKD based on a single measurement and should always be interpreted in context of the patient, as values tend to fluctuate daily. Persistently elevated values of both these biomarkers are sufficient for diagnose CKD. For such evaluation, it is worth noting that the patient should be previously fasted, hydrated and stable. Besides, at least two measurement should be performed every time kidney function is assessed (Groves, 2020; *IRIS Kidney - Guidelines - IRIS Staging of CKD*, 2023).

CKD is divided into four different stages (Table 1). Stage I patients present without azotaemia, however some other renal abnormalities like inadequate urinary concentration capacity without identifiable non-renal cause (found in cats, not really reported in dogs), abnormal renal palpation and imaging findings or persistent elevated (> 14mg/dL) SDMA concentrations

may be identified. Stage II patients can present either normal or mildly increased creatinine and mildly increased SDMA, with or without the manifestation of clinical signs. Stage III patients have moderate azotaemia and present extrarenal signs, which vary in extent and severity. Finally, stage IV patients will have severe azotaemia with increased risk of systemic signs and uremic crises (*IRIS Kidney - Guidelines - IRIS Staging of CKD*, 2023).

Table 28 - Staging of CKD based on blood creatinine and SDMA concentrations in cats. Source: (IRIS Kidney - Guidelines - IRIS Staging of CKD, 2023)

Stage	Blood creatinine (µmol/L)	SDMA (µg/dL)
1	<140	<18
2	140-250	18-25
3	251-440	26-38
4	>440	>38

Substaging is performed by measuring urine protein:creatinine ratio (UPC) and blood pressure. UPC should be measured in all CKD patients, if UTI or haemorrhage are absent, and it should be done over a period of at least two weeks, with a minimum of two urine samples (Table 2). Non-proteinuric or borderline proteinuric can be categorized as microalbuminuric (*IRIS Kidney - Guidelines - IRIS Staging of CKD*, 2023). Microalbuminuria is defined as the presence of albumin in the urine, however its significance in predicting CKD is still unknown and it is not necessarily associated with CKD, therefore the underlying cause needs to be defined firstly (Paepe & Daminet, 2013).

Table 29 - Substaging by proteinuria in cats. Source: (IRIS Kidney - Guidelines - IRIS Staging of CKD, 2023)

UP/C value	Substage
<0.2	Non-proteinuric
0.2-0.5	Borderline proteinuric
>0.5	Proteinuric

At least five blood pressure measurements should be done in calm patients and without sedation (Chen *et al.*, 2020). Afterwards, the patient is substaged according to systolic blood pressure and risk of target organ damage.

Risk of organ damage can be classified as minimal, low, moderate or high, depending on how much the systolic pressure increases above the breed-specific reference range and animals can be classified as normotensive, prehypertensive, hypertensive and severely hypertensive, according to these values (Table 3). Systolic blood pressure measurements should be done, at the very least, two hours apart from each other (*IRIS Kidney - Guidelines - IRIS Staging of CKD*, 2023).

Table 30 - Substaging by blood pressure. Source: (IRIS Kidney - Guidelines - IRIS Staging of CKD, 2023)

Systolic blood pressure (mmHg)	Substage	Risk of future target organ damage
<140	Normotensive	Minimal
140-159	Prehypertensive	Low
160-179	Hypertensive	Moderate
≥180	Severely hypertensive	High

Besides the previously mentioned creatinine, urea and SDMA, other parameters like serum phosphate and ionized calcium may assist diagnosis and help predict disease progression and prognosis, together with the hemogram, urinalysis and ionogram.

4.4.3.2. Ionogram

Regarding that the effects phosphate has on the progression of CKD are well known and its relation with PTH, measurement of phosphate may be useful to predict secondary renal hyperparathyroidism (Langston, 2017). Phosphate retention and hyperphosphatemia (serum phosphate levels greater than 4.5 mg/dL) occur primarily due to impaired renal excretion and this will, in turn, interact with ionized calcium and calcitriol levels, stimulating PTH secretion. Increases in serum levels have been associated with increased mortality in cats with CKD. According to IRIS, it is recommended to keep serum phosphate levels between 2.5-4.5 mg/dL in IRIS stage II, 2.5-5 mg/dL in stage III, and 2.5-6 mg/dL in stage IV cats (Kidder & Chew, 2009; *IRIS Kidney - Guidelines - IRIS Staging of CKD*, 2023).

With CKD the serum concentration of sodium may vary, as most cats usually present normonatraemic or hyponatraemic due to dilution caused by fluid retention. Hypernatraemia may occur, although infrequently, due to neurological (reduced mentation and mobility) and urological diseases, which corresponds to an increase in mortality rates, as it also correlates with dehydration and hypovolaemia. Hypernatraemia is also associated with neurological signs, as a consequence of water movement out of brain cells, causing the rupture of cerebral veins. Some other clinical signs are vomiting, ataxia, lethargy, and weakness (Ueda, Hopper & Epstein, 2015).

Chloride changes tend to correlate with sodium changes and can be used to determine the acid-base status in CKD cats (Elliott *et al.*, 2003; Langston, 2017). Most commonly cats present normal chloride levels, however acidosis can occur due to a reduction on bicarbonate concentrations and an increase in the anion gap. Hyperchloremia may occur in cats due to more specific tubular dysfunction (proximal renal tubular acidosis) or when bicarbonate is overdosed to buffer excess acid formed in the body. In both of these situations, chloride is reabsorbed in place of bicarbonate, leading to acidosis (Elliott *et al.*, 2003). For reference, normal ranges for feline chloride and sodium concentrations are 117-123 mEq/L and 147-156 mEq/L, respectively (Campbell & Chapman, 2000).

Hypokalaemia and potassium depletion are common findings in cats with CKD stages II and III, while stage IV patients tend to develop hyperkalaemia due to potassium retention as a consequence of extremely low GFR. Although the cause of hypokalaemia in cats has not been

fully clarified, it was established that dietary potassium deficiency, increased urinary loss and RAAS activation are the most likely perpetrators. In addition, metabolic acidosis, commonly concurrent to CKD, causes intracellular K^+ to shift into the plasma and, consequently, reduces the body stores of this electrolyte and masks hypokalaemia until they become depleted (Scherk & Laflamme, 2016). Such depletion may lead to hypokalaemic myopathy (identified with ventroflexion and plantigrade stance), progression of kidney injury and polyuria/polydipsia (PU/PD), muscle weakness and lethargy, which may further enhance the acidotic state if severe hypokalaemia (<2.5 mmol/L) is present. In contrast, mild hypokalaemia (2.5 – 3.0 mmol/L) is often asymptomatic. (Polzin, 2011, 2013; Reynolds & Lefebvre, 2013; Quimby, 2016; Sparkes *et al.*, 2016).

Magnesium abnormalities may also be present in these cases in the form of hypomagnesaemia or hypermagnesaemia, although the latter is more common. Both of these are associated with increased mortality and may be related to nephrolithiasis in cats with CKD, having an increased prevalence in these sorts of cases. Reference range for total serum magnesium is 1.58-2.16 mg/dL. (Chacar *et al.*, 2019).

Calcium, as previously mentioned, may be heavily influenced by phosphate, PTH and calcitriol concentrations, resulting in hypercalcaemia which can then lead to soft tissue calcification, further decline of renal function and worsening of azotaemia. Besides this, formation of calcium oxalate uroliths is promoted by excess calcium, which may lead to urinary obstruction. Furthermore, some studies suggest that hypercalcaemia may have a role in pancreatitis, however it is still uncertain. Hypercalcaemia is defined by a total calcium concentration greater than 11 mg/mL in cats and is confirmed by measuring the ionized calcium concentrations over 1.4 mmol/L. With more advanced CKD it is possible to see hypocalcaemia when total calcium concentrations are below 9 mg/dL or ionized calcium concentrations are under 1.2 mmol/L (Odunayo, 2014; Finch, 2016; Langston, 2017; Broek, van den *et al.*, 2022).

Functional iron deficiency is a potential contributor to anaemia, and it is common in CKD cases. This occurs secondary to reduced intake, decreased absorption or bleeding through the gastrointestinal tract. The suggested mean to determine the existence of this deficiency is by evaluating the percent transferrin saturation (TSAT) and ferritin concentration. According to Betting *et al.* (2022), cats with TSAT values under 20% are considerate iron deficient. However, ferritin is not considered and accurate tool for investigating functional iron deficiency in cats, because an wide range of values (from 138 to 838 ng/mL) can be presented in healthy cats, making it hard to diagnose with this marker (Betting *et al.*, 2022; Gest *et al.*, 2015).

4.4.3.3. Hemogram

Anaemia is a common finding in cats with CKD, usually being normochromic, normocytic and hypoproliferative, therefore being a non-regenerative anaemia. It is defined as a packed cell volume (PCV) below 25% (normal reference range in cats is 25-45% (Tasker, 2012)). Furthermore, this complication can be confirmed through the determination of haemoglobin, which due to a possible impairment of iron utilization will be decreased, and total serum proteins

or total plasma proteins, that help to differentiate between anaemia due to blood loss (low to normal protein) or haemolysis (normal to high protein). Blood smears are also an important diagnostic tool, allowing to access cell morphology, platelet count and leukocyte differentials, as well as identifying the type of anaemia. Mean cell volume, red cell distribution width and mean cell haemoglobin concentration are other parameters that can be used to determine the type (regenerative or non-regenerative), usually remaining within the reference values in CKD induced anaemia. Reticulocyte counts are also used to differentiate between regenerative and non-regenerative anaemia, being that in CKD, due to the reduction in EPO, reticulocyte counts are often lower (Tasker, 2006, 2012).

4.4.3.4. Urinalysis

USG is routinely measured in veterinary medicine using a refractometer. Normal range is, according to IRIS, from 1.035 to 1.060 in healthy cats that are normally hydrated. Very concentrated urine may appear when there is reduced renal perfusion or in cats eating mainly dry food without consuming enough water. Isostenuric or hypostenuric urine appears when healthy animals excrete surplus fluid to maintain homeostasis, however it may also be present (USG <1.008) in diabetes insipidus and conditions that cause tubular insensitivity to ADH. Therefore, further investigation should be made in these cases. In addition, moderately concentrated urine, with USG of 1.034 are present in healthy animals, and only those that fail to produce concentrated urine require further investigation (water deprivation and/or vasopressin administration, for example) (Watson *et al.,* 2022).

Most cats with CKD become isosthenuric (USG 1.008-1.012), however it is also possible to see some with USG values as high as 1.040 or 1.045 where CKD can still be suspected, as long as persistent azotaemia is present. Kidney disease may also cause inappropriately concentrated urine in over-hydrated patients, exceeding values of 1.007, and inappropriately diluted urine in dehydrated patients, with values below 1.035 present, which requires further investigation to discard other abnormalities (Paepe & Daminet, 2013; Watson *et al.*, 2022).

Besides USG, the presence of proteinuria is also valuable to identify and monitor in CKD patients. If the UPC is above 0.4 its origin should be evaluated (pre-renal, renal and/or post-renal) and a stepwise diagnostic approach should be made to eliminate other causes of proteinuria not related with kidney disease (for instance, stress, systemic inflammation, hypertension). Routine tests for proteinuria are available as dipsticks, however they are only truly reliable to detect severe proteinuria in cats, therefore false positives and false negatives are common in these tests. Hence, to confirm results, a more sensitive and specific test is available, the sulfosalicylic acid turbidity test, which allows to confirm positive results (Paepe & Daminet, 2013; *IRIS Kidney - Guidelines - IRIS Staging of CKD*, 2023).

Microalbuminuria may also be used to clarify suspected false-negative dipstick results, by measuring the urinary albumin:creatinine ratio with an enzyme-linked immunosorbent assay (ELISA). However, microalbuminuria is not used routinely, beacuse it may be related with other diseases, lacking in benefit over UPC. Furthermore, a negative result does not necessarily rule

out proteinuria. Nevertheless, there are still some specific situations where it may be recommended to use this test (Paepe & Daminet, 2013). Bacterial cultures are also recommended as routine during CKD follow-ups, as there is an increased risk of UTIs (Paepe & Daminet, 2013).

The collection of urine for testing should be made, either by natural voiding or manual compression of the bladder, transurethral catheterization or cystocentesis, in order to obtain the highest quality sample with the least risk for the patient. Ideally, urine collection is done early in the morning in a fasted patient and collected by cystocentesis, as it avoids/reduces contamination of the sample (Hüttig & Roura, 2022).

4.4.4. Complementary diagnostic

4.4.4.1. Imaging

There are two practical methods of analysing the kidneys in CKD patients: x-ray and ultrasound. X-ray is best suited to quickly compare kidney sizes and shape, allowing the identification of radiopaque calculi in the urinary tract. For optimal radiographic assessment of the kidneys, a ventrodorsal and a lateral projections should be attained (Pollard & Phillips, 2017).

With the ultrasound, it is possible not only to assess the shape and size of the kidneys, but also to evaluate the cortex, medulla, overall echogenicity of the organ, which may be diffuse or focal, and, in some cases, to identify mineralization of the tissue. Although in end-stage disease, it may be difficult to identify the renal tissue, due to the changes it undergoes, ultrasound is still the preferred imaging tool to assess the kidneys' morphology (Pollard & Phillips, 2017).

4.4.4.2. Biopsy

Renal biopsy is only indicated in cases whose results are likely to establish a definitive diagnosis, determine the severity of the lesion and facilitate prognostics and to work out an optimal treatment plan. This method serves as a definitive diagnosis of glomerular disease, but it may be unlikely to benefit the patient if end-stage kidney disease is already present, considering that once such stage is established, a definitive diagnosis will not alter the prognosis nor the outcome of the disease. Therefore, some considerations should be made before conducting the procedure, especially in advanced CKD patients, where renal biopsy is contraindicated, because there is an increased risk of complications, which outweighs the necessity for a definitive diagnosis. Other contraindications to renal biopsy include severe anaemia, uncontrolled hypertension, extensive pyelonephritis, hydronephrosis, and kidney cysts (Vaden, 2005; Paepe & Daminet, 2013; Fallois, de *et al.*, 2022).

There are several biopsy technics, however the needle used for sample collection should always remain in the renal cortex and should not cross the corticomedullary junction (Vaden, 2005; Fallois, de *et al.*, 2022).

4.5. Concomitant diseases

4.5.1. Hyperthyroidism

Hyperthyroidism is commonly found in CKD patients, and it is important to consider both conditions together, as they are prevalent in older cats, as well as sharing a number of historical findings and clinical signs. In addition, hyperthyroidism may “mask” the presence of CKD due to the effect of the hyperthyroid state in the kidneys and cardiovascular system, lowering both serum creatinine and SDMA concentrations, by increasing GFR and decreasing muscle mass, due to the increases in triiodothyronine, which causes vascular smooth muscle relaxation, reducing peripheral vascular resistance and consequently activating the RAAS. Furthermore, CKD may lead to a mild suppression of the thyroid hormone’s concentration, as non-thyroidal diseases supress circulating thyroid hormone concentrations, making it more difficult to diagnose. It is also stated that hyperthyroidism is involved in the progression of renal disease, although it is still not certain, but it may be related to altered renal haemodynamic, hyperfiltration and the increases in proteinuria identified in hyperthyroid states. Any changes in GFR due to hyperthyroidism will be reversed once euthyroidism is re-established, causing its decrease and, as a consequence, creatinine levels increase. It is noteworthy to know that when treating patients with both illnesses, priority should be given in treating hyperthyroidism first, considering that only after the thyroid levels normalize it is possible to assess and correlate creatinine and urea levels, to better identify and understand CKD in cats (Jepson, 2016; Geddes & Aguiar, 2022; Yu *et al.*, 2022). This treatment should also be addressed with caution, due to the possibility of worsening renal function, which in turn would result in the exacerbation of uraemic signs. (Yu *et al.*, 2022).

4.5.2. Urinary tract infection

UTIs are relatively common in cats diagnosed with CKD, with older female cats being more susceptible (Sparkes *et al.*, 2016). Some authors show concerns regarding the progression of CKD or the development of an acute on chronic kidney disease due to the possibility of ascending infections that may result in either acute or chronic pyelonephritis (Mayer-Roenne *et al.*, 2007; Jepson, 2016). It has been hypothesised that lower USG could be related with the occurrence of UTIs in cats with CKD, however this has still not been proven in feline patients, being the most likely cause the impairment of normal host defence mechanisms, which in turn allows bacteria to colonize the lower urinary tract. (Mayer-Roenne *et al.*, 2007; Bailiff *et al.*, 2008).

4.5.3. Ureterolithiasis

Upper urolithiasis is another common concurrent disease in cats with CKD, and it is considered to be a potential cause of CKD when occurring unilaterally and when there is no pre-existing renal disease (Jepson, 2016; Hsu *et al.*, 2022). In these cases, cats fed exclusively dry food and urine-acidifying food, usually used in UTI treatment, are more likely to develop uroliths in the upper urinary tract. Other risk factors include overly concentrated urine, which can be masked by CKD, through the kidneys inability to concentrate urine. Consequently, feeding wet

food may be beneficial to prevent urolithiasis recurrence, which could contribute for the progression of CKD. A study conducted by (Hsu *et al.*, 2022) also revealed that purebred cats are more likely to develop calcium uroliths and hypercalciuria, as they present elevated levels of ionized calcium, a consequence of the CKD or due to genetic predisposition (Hsu *et al.*, 2022).

4.5.4. Inflammatory bowel disease

It is hypothesised that longstanding feline inflammatory bowel disease might enhance the systemic inflammatory burden, allowing passage of molecules that would normally be retained in the intestinal tract, and eventually end up in the kidney, which in turn may be a promoter of CKD (Brown *et al.*, 2016).

4.5.5. Diabetes *mellitus*

Diabetes Mellitus is considered the main cause of CKD in humans, however its relation with CKD in cats is still unknown. Some studies reveal no visible correlation between the two diseases, although admittedly it remains possible that diabetes in a CKD cat might correlate with shorter life expectancy (Jepson, 2016). A study mentioned by Yu *et al.* (2022) reveals that no significant difference was found in renal parameters of cats with poorly controlled diabetes and the healthy ones. Another paper published by Pérez-López *et al.* (2019) in a large population of cats with CKD revealed that a higher frequency of diabetic cats had CKD, which could mean that the renal disease would be a complication of the diabetes *mellitus*, however more studies should be made in order to get to a conclusion.

4.5.6. Periodontal disease

Periodontal disease is a common but preventable disease in older cats whose prevalence and severity are established as risk factors for CKD. It is associated with not only local disease of the oral tissues but also with systemic effects. Furthermore, it is believed that the production of inflammatory cytokines, or endotoxemia, and immune responses to bacterial/viral infections are the main mechanisms by which periodontal disease may cause CKD. In addition, the effect of the pharmaceuticals used to control dental disease, such as antibiotics and NSAIDs, or general anaesthesia for a dental procedure may be potential collaborators to kidney injury. On the other hand, it is also believed that dental cleaning has the potential to improve kidney function in animals during early CKD, as long as there are no other complications (Finch, Syme & Elliott, 2016; Hall *et al.*, 2021).

4.6. Control and Treatment of CKD

Currently, the strategy for dealing with CKD in cats relies mostly on supportive and symptomatic treatment, aiming to improve the patient's quality of life and, when possible, slowing down the progression of the disease. When planning the patients' medical management, it is important to recognize and treat active renal disease (Polzin, 2011; Taylor & Sparkes, 2013; Sparkes *et al.*, 2016). Adequate food intake is also an important factor to consider in cats with

CKD, hence treatment should address underlying causes for poor appetite (i.e., acidosis, anaemia, hypertension), so it can correct negative balances in both energy and protein levels (Larsen, 2016). The treatment plan should be made considering the patient's needs, hence a thorough examination (medical history, physical exam, complete blood count, biochemistry profile, urinalysis, blood pressure measurements, UPC and imaging) is a must (Polzin, 2013).

Moreover, it is also important to have in mind the pet owner's time and resources, as they are key factors for the success of the therapeutic plan. Hence, the owner should be informed about the nature of the disease, duration and purpose of the treatment. Likewise, planning an active follow-up of the patient in advance might help with owner compliance. The more compliant the owner is, the more successful is the control over the CKD (Polzin, 2013).

4.6.1. Diet

Diet is typically the standard method of controlling CKD, being important to maintain the patient's body weight and muscle mass, halting the progression of the disease. Typically, renal diets have less protein, phosphorus and sodium content when compared with commercial diets, and also have more caloric density, potassium, vitamins, antioxidants, and omega-3 polyunsaturated fatty acids (Machado *et al.*, 2022). Although renal diets remain controversial for stage I patients, they have been proven to be beneficial to cats with CKD stage II to IV. Several clinical trials reveal that renal diets delay the onset of uraemia and also maintain or improve nutrition and quality of life. The transition into renal diet should be gradual, throughout several weeks and by mixing increasing amounts of the new diet to the old one. Moreover, diet changes should be done only once metabolic, gastrointestinal, or dental complications are resolved so that it does not promote a dietary aversion.

"Renal" formulated diets typically have somewhere between 6-7 g of protein per 100 kcal, however it should be noted that older cats (over 13 years old) may have increased needs and severe restriction of protein may lead to muscle mass loss, thus moderation is required (Sparkes *et al.*, 2016).

There are pro and con views regarding the role of dietary protein in the management of feline CKD, as there is insufficient clinical data to provide guidance on both inadequate and excessive protein intake. The encouraged attitude is to have a more conservative approach for the diets bestowed to CKD cats, in order to avoid providing too little protein, which may result in decreased quality of life as the dietary requirements are not met, and refrain from excessive intake, which increases the risk of morbidity and uraemia (Larsen, 2016; Polzin & Churchill, 2016; Scherk & Laflamme, 2016; Yu *et al.,* 2022)

Body condition score, body weight, caloric intake, serum albumin, PCV and quality of life should all be monitored once the renal diet starts. Moreover, patients unable to meet the nutritional goals should be evaluated for uraemia, dehydration, blood and electrolyte abnormalities, UTIs, progression of CKD and non-renal diseases (Polzin, 2011, 2013).

A study was conducted by Schauf *et al.* (2021) with the end goal of investigating the response of cats with early CKD to varying protein, phosphorus and calcium:phosphorus ratios. In this study, a commercial clinical renal diet containing low protein and low phosphorus, and another containing moderate protein and phosphorus, were given to two different cat populations. Upon comparing the long-term effects of each diet, the authors concluded that, when feeding a dry diet with highly restricted protein and phosphorus and a high calcium:phosphorus ratio, cats with early CKD had controlled azotaemia but developed hypercalcemia in the majority of cases, which also resulted in increased plasma FGF-23, SDMA and frequency of urolithiasis. Oppositely, a dry-wet diet with moderately restricted protein and phosphorus and lower calcium:phosphorus ratio was able to prevent hypercalcemia and azotaemia.

4.6.2. Phosphorus

The first step for controlling the progression of CKD is to reduce phosphate retention by controlling the diet phosphorus content, which should be maintained according to specific ranges recommended by the IRIS guidelines (Polzin, 2011). Furthermore, this is a recommended procedure in cats with CKD stages II to IV, and to achieve it the patient needs to be rehydrated, have their dietary phosphorus restricted. In addition, the use of phosphate binding agents (PBA), like sodium bicarbonate and potassium citrate, may be necessary in some cases or when the treatment fails to achieve target concentrations after 4 to 8 weeks (Korman & White, 2013; Polzin, 2013). Diet alone may be capable of decreasing PTH and FGF-23 or even preventing its increase in the early stages of kidney disease, however it may be inefficient as the CKD advances. In dogs, the use of calcitriol may be required to normalize PTH, however its use as not been proven to be beneficial in feline patients (Brito Galvao *et al.*, 2013).

Although its efficacy has not been consistent in feline patients, a study where pulse dosing of calcitriol twice weekly at 3.5 times the daily dose (2.5 to 3.5 ng/kg) showed to suppress PTH and, consequently, phosphate. In order to avoid complications associated with hypercalcaemia, once PTH stabilises within the reference range for at least two to three months, pulse dosing can be reduced to a twice weekly daily dosing. Therefore, special attention should be given to calcium and PTH levels while the treatment is ongoing (Brito Galvao *et al.*, 2013).

In veterinary medicine, the most commonly employed PBAs contain aluminium, which are the most widely employed, and calcium (Kidder & Chew, 2009). Aluminium-containing agents like aluminium hydroxide, aluminium carbonate and aluminium oxide have a recommended starting dose of 30 to 100 mg/kg/day, while calcium-based agents have a recommended starting dose of 60 to 90 mg/kg/day if calcium acetate is used, and 90 to 150 mg/kg/day for calcium carbonate. However, these calcium-based products may promote hypercalcaemia, therefore serum calcium should be monitored when using these drugs. A new PBA called lanthanum carbonate also seems quite effective in reducing phosphorus absorption in the intestines, resulting in minimal side effects. The initial dose of this product is 30 mg/kg/day (Polzin, 2011).

Regardless of the PBA used, they should be dosed to “effect”, or, in other words, adjusted until the therapeutic target is reached (Polzin, 2013).

4.6.3. Protein

Proteinuria should be managed in all CKD patients when UPC ratios exceed 0.4 in cats with CKD stages II, III and IV, or 2.0 in cats with CKD stage I (Geddes & Aguiar, 2022; Polzin, 2011). Standard therapy used for proteinuria involves dietary restriction of protein and the use of angiotensin converting enzyme inhibitors (ACEI) or angiotensin receptor blockers (ARB) with the goal of lowering UPC ratios to at least half (Polzin, 2013).

Initial dose of enalapril and benazepril, the most widely used ACEI drugs, are 2 mg/kg/day and 0.25 to 0.5 mg/kg given orally every 12 to 24 hours respectively. Benazepril is preferred due to its largely hepatic clearance rather than renal excretion, which is the case of enalapril. It is also important to monitor potassium levels when doing an ACEI treatment, as they can cause hyperkalaemia (Polzin, 2011).

Initial dose for telmisartan, a very effective and the most widely used ARB in dogs and cats, has an initial dose of 1 mg/kg/day given orally (Sent *et al.*, 2015).

4.6.4. Potassium

Oral potassium supplementation is recommended in hypokalaemic stable feline patients. It may be provided as potassium gluconate at a dose of 1 to 4 mEq per cat twice a day or potassium citrate at a dose of 40 to 75 mg/kg divided, twice a day. The latter has the added advantage of being an alkalinizing agent, however there is no data of its effectiveness in metabolic acidosis. In decompensated patients, intravenous administration of potassium chloride may be given at a rate of 0.4 mEq/kg/h, and potassium should be monitored every 3 to 6 hours. Potassium chloride may also be given subcutaneously at a concentration up to 30 mEq/L. However, it may cause irritation if administered this way. If hypokalaemic myopathy is present, it should resolve within one to five days after starting oral or parenteral treatment (Quimby, 2016).

4.6.5. Gastrointestinal clinical signs

In patients with gastrointestinal signs, like anorexia, nausea and vomiting, management should include H2 blockers or proton-pump inhibitors, antiemetic drugs, and sucralfate (Polzin, 2013). According to Batchelor (2012), the most effective antiemetics for cats appear to be maropitant, ondansetron, although oral maropitant is used off-label in cats, and metoclopramide. The latter is considered a first-line antiemetic for this specie, even though its use as a central antiemetic is questionable, at a dose of 0.2 to 0.5 mg/kg PO or subcutaneously, every eight hours (MSD Veterinary Manual, 2023). The registered dose of maropitant in Europe is 1 mg/kg in cats for up to 5 days by subcutaneous injection (Jonathan Elliot, 2022).

Another study compared the effects of omeprazole, a proton-pump inhibitor, on the gastric pH and determined that it had an effect superior to that of famotidine, an H2 blocker, at a dose of 1 mg/kg PO twice a day (Quimby, 2016).

In anorectic patients, the use of nasoesophageal, oesophageal or gastrotomy tubes depends on the type and duration of feeding desired, being the oesophageal tube the most commonly used. These are of great value for long-term management of CKD, as they grant easy access for food, water and medication, being usually well tolerated by the patients and allow for less stressing care (Quimby, 2016; Ross, 2016).

In animals that develop constipation, an enema therapy or manual deobstipation, under anaesthesia, which is suboptimal in a uremic crisis, may be required. However, before any action is taken, it is necessary to correct ongoing dehydration and hypokalaemia. A common enema formula used in clinic consists of warm water, sterile lubricant and dioctyl sodium sulfosuccinate (pet-enema) administered slowly through a feeding tube at a rate of 5 to 10 mL/kg and repeated every 8 to 12 hours. Laxatives are also a major part of the management of constipation once the imbalances previously mentioned are corrected (Quimby, 2016).

For patients that develop ulcerations in the oral cavity, topical treatment with chlorhexidine-containing oral rinses have been proven to be useful in their management. However, if the patient remains hyporexic or anorexic, despite appropriate routine care, the addition of appetite stimulates, like mirtazapine, at a dose of 1.88 mg/cat orally every 48 hours for three weeks or as a transdermal formulation, at about 2 mg/cat every 24 hours for two weeks (Scherk & Laflamme, 2016; Elliot, 2022), may be helpful in increasing caloric intake, together with antinausea medication (Ross, 2016; Elliot, 2022). Prebiotics may also be of use, having shown to improve microbiota activity and minimize the accumulation of uremic toxins (Hall *et al.*, 2022).

4.6.6. Fluid therapy

Fluid therapy is recommended in CKD patients, which usually appear dehydrated in consult. This can be done through a balanced electrolyte solution (i.e., Ringer's Lactate) either intravenously, to quickly rehydrate the patients, or subcutaneously at a volume of 75 to 100 mL per dose, depending on the patient's size, when treating recurrent or chronic dehydration. Moreover, as a more conservative approach, water supply either by feeding tube, the use of moist feed or the employment of fountains is important, as the ingestion of water avoids sodium increases associated with the subcutaneous fluid injection, which, as evidence suggests, may be harmful to the kidneys and may impair the effectiveness of antihypertensive therapy (Korman & White, 2013; Polzin, 2011).

Acidosis can be improved with the renal diet alone, due to its neutral pH. However, when diet alone is not enough, administration of sodium bicarbonate or potassium citrate is indicated, with the latter offering the advantage of treating hypokalaemia as well as acidosis. The starting dose is 40 to 60 mg/kg every 8-12 hours for potassium citrate and 8 to 12 mg/kg every 8-12 hours for sodium bicarbonate. After initiating therapy, blood gas analysis should be performed every 10 to 14 days and the dose adjusted accordingly until the values normalize (Polzin, 2011).

4.6.7. Anaemia

When anaemia is present, it is necessary to first address all factors that contribute to it, so that optimum therapeutic response can be achieved. Anabolic steroids, like nandrolone, stanozolol and cypionate, can be used in the treatment of anaemia, resulting in the increase of haematocrit values, appetite and muscle mass. However, their use is not currently recommended for routine care due to lacking information regarding efficacy and their potential hepatotoxic effect (Polzin, 2013; Sparkes *et al.*, 2016). The use of erythropoietin-stimulating agents, like epoetin and darbepoetin (Figure 12), should be considered when anaemia is persistent and symptomatic, as these pharmaceuticals have proven to be beneficial in improving appetite and quality of life (Korman & White, 2013). In addition, the use of these agents is recommended if the PCV is persistently low. Repeated monthly monitoring, together with reticulocyte count and blood pressure should be made until the PCV values normalise. If the response to the therapy is poor, then iron status should be checked and the patient re-examined to exclude the presence of any concomitant disease (Sparkes *et al.*, 2016). Iron supplementation is also recommended when using erythropoietin-stimulating agents, due to the process having a high demand in iron.

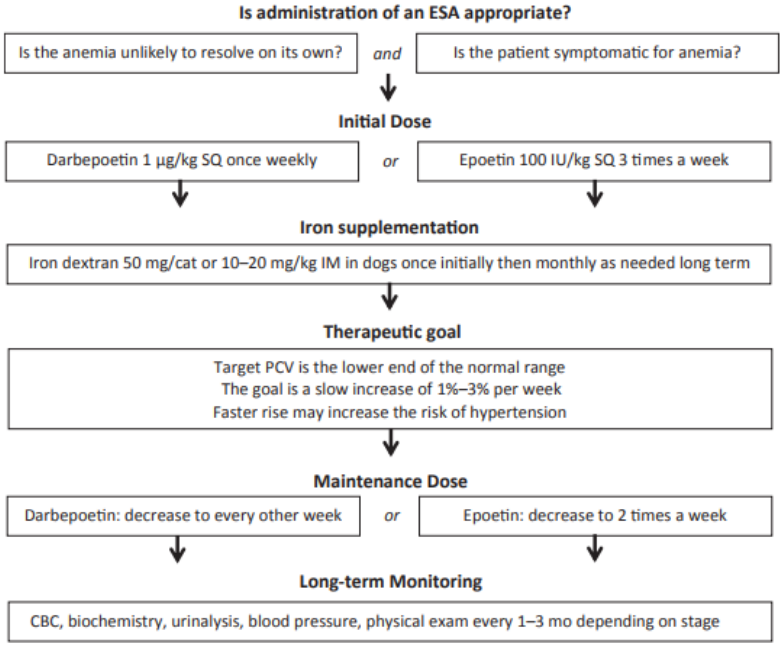


Figure 12 - Anaemia treatment with erythropoietin-stimulating agents

4.6.8. Hypertension

Hypertension is another factor that should be resolved as soon as possible, in order to minimize the negative effects and improve survivability (Lawson & Jepson, 2021). Suppression of the RAAS using angiotensin converting enzyme inhibitors (ACEI) or angiotensin receptor blockers (ARBs) has been demonstrated to halt renal fibrosis (Lawson *et al.*, 2015). Aldosterone inhibition is another possibility, by either using a nonselective agent like spironolactone or by using a aldosterone receptor inhibitor such as eplerenone (Jepson, 2016). While the first is useful for lowering mortality rates when cardiomyopathy is present, the latter is more effective in treating

hypertension due to vasoconstriction. Eplerenone is administered at 2.5 to 5 mg/kg *per os* once a day, with the reports of its use indicating prolonged survival times and lower BUN values (Kai, Ohishi & Hikasa, 2022).

Amlodipine, a calcium channel blocker, has shown to improve cats' quality of life due to being an effective antihypertensive and antiproteinuric agent in cats. For that reason, it is considered the drug of choice for treating hypertension in this species (Korman & White, 2013; Polzin, 2013). Initial doses are 0.625 mg for cats under 5 kg and 1.25 for cats over 5 kg (Quimby, 2016; Sparkes *et al.*, 2016).

4.6.9. Mesenchymal stem cell treatment

A newer form of treatment that is being researched is the mesenchymal stem cell (MSC) treatment. Across all studies in cats, a trend towards improvement in kidney function and GFR, as well as stabilization of body weight by enhancing diet and water consumption has been verified with this new approach. Although, a single study has revealed that defrosted and administered cryopreserved MSC caused an instant blood mediated inflammatory reaction, overall, this therapy is well tolerated. Hence, MSC therapy may be a new effective approach to slowing down renal disease and improve renal function in CKD patients (Quimby *et al.*, 2013; Zacharias *et al.*, 2021).

4.7. Monitoring

After initiating treatment, CKD patients should be routinely reassessed, through the analysis of serial serum BUN, creatinine, and electrolytes, especially phosphate and total calcium, as they are important to detect the development of hypercalcaemia or hyperphosphatemia, in particular when vitamin D and activated vitamin D compounds are being used. This can provide information about the progression of the CKD.

As CKD progresses, the patients´ needs may vary, hence the importance of continuously monitoring. In one hand, patients with stages I and II require less frequent check-up consults compared with later stages of the disease, being recommended to monitor them every four to six months once renal function stabilizes. On the other hand, both stable patients with stages III and IV kidney disease should be re-seen every three to four months. In the case of CKD stage IV patients with ongoing gastrointestinal signs or that have recently recovered from a uremic crisis it is recommended to recheck them, at least, within two to five days after being discharged. (Polzin, 2013).

Besides, if proteinuria and hypertension are present, frequent monitorization is crucial. Haematocrit, urinalysis, UPC and a thorough physical exam and nutritional assessment should also be performed, as loss of body mass can be a predictor of mortality (Polzin, 2011; Brito Galvao *et al.*, 2013; Polzin & Churchill, 2016; Scherk & Laflamme, 2016).

Prophylactically, healthy cats that are considered at risk of developing CKD and hypertension due to their life stage should also be kept under surveillance. The recommendation is that cats aged seven to ten years should be seen at least once every year and cats aged eleven

years or above every six to twelve months. This proactive approach allows for a better control of the disease, preventing morbidity and increasing the chances of survival and quality of life (Lawson & Jepson, 2021).

IV. Clinical cases

1. Patient “Chivers”

“Chivers” was an indoor cat with outdoor access, rescued in 2010, with 15 years of age. When rescued, the cat already came neutered, vaccinated and dewormed. He presented on consult at Donaldson’s Vets in 2021 because the owner noticed some changes in the cat’s behaviour.

1.1. Previous clinical history

In 2021, “Chivers” was diagnosed with medial patellar luxation in the left hind and was scheduled for surgery (wedge sulcoplasty) the next day. However, the surgery came with complications, meaning that he needed to stay under anaesthesia for a prolonged amount of time. The day after, “Chivers” was discharged, as he appeared to be doing well, despite not passing any urine while in hospital. Three days after the discharge, he started vomiting and still did not urinate. When he got back to the practice, blood analysis revealed severe azotaemia and elevated SDMA. AKI was diagnosed and “Chivers” was again admitted for intravenous infusion at 5 mL/kg/h until he started passing urine and his azotaemia stabilised. However, kidney values continued fluctuating even when hydrated, being made the diagnosis of CKD.

1.1.1. AKI

When AKI was diagnosed, “Chivers” had creatinine values of 686 µmol/L, urea values of 39.8 mmol/L, SDMA values of 44 µg/dL and phosphate at 4.04 mmol/L, with no other detectable changes in his biochemistry and electrolyte values. When hospitalized, the cat was put on intravenous fluids at a rate of 5 mL/kg/h and monitored. The day after the hospital admit, “Chivers” started passing urine again and started eating as well as a noticeable improvement in serum urea and creatinine levels, going as low as 249 µmol/L for creatinine and 13.7 mmol/L for urea. A slight hypercalcaemia could also be seen, varying between 166 and 167 nmol/L, and resolving after reducing the intravenous fluids rate. Although noticeable, these improvements were still not enough to bring the values back into the reference range, as these kept persistently above them regardless of the fluid therapy. Hence the diagnosis change from AKI to CKD.

1.2. Managing and monitoring CKD

After the update on diagnosis, the patient medication included the administration of benazepril, at one 2.5 mg tablet once a day, as well as lowering fluid therapy rates to 2 mL/kg/h.

After two more days, renal figures plateau around 274 µmol/L and 19.1 mmol/L for creatinine and urea respectively, with SDMA also around 15 µg/dL at that time, being then sent home on renal diet (SPECIFIC® cat FKD kidney support) and benazepril. For the next few days constant monitoring would be kept on “Chivers”, being observed every two days for appraisal of renal figures, which did not vary much since the last stay in hospital, with one or two episodes of vomiting, low appetite, and hard dry stools. Maropitant and lactulose were given to reverse the gastrointestinal signs, which seemed to resolve for the time being.

Monitoring consults became less frequent, as the patient remained stable, and azotaemia values did not fluctuate significantly. Weight maintained, as the owner is monitoring both food and water intake in “Chivers”, ensuring that he always has available water and that he drinks a minimum of 200 to 250 mL of water per day (about 50 mL/kg/day). By the end of the year, after constant blood works done to monitor the condition, “Chivers” started to gain some weight, not showing any symptoms of kidney disease. Next year, in 2022, “Chivers” was only monitored twice per month, being that renal values remained unremarkable throughout the time period.

From October 2022 to March 2023, the patient was accompanied by the author, where more monitoring tests were made, including full haematology and biochemistry screening, including electrolytes. In November “Chivers” maintained for the first time blood urea and creatinine levels within the reference range, despite developing some dental disease and losing some weight due to having less appetite. Near the holidays, the patient had an episode of vomiting and a reduction in food and water consumption.

Blood tests discovered a mild non-regenerative anaemia (PCV of 32%) and a moderate increase in the renal parameters since the last measurement (creatinine at 268 µmol/L and urea at 15.2 mmol/L). As a consequence, the patient was admitted again for intravenous fluid therapy and monitoring. Fluid therapy had to be reduced to 2 mL/kg/h as blood pressure was high (181/122), and amlodipine 1.25 mg (half a tablet) was given to help and normalize it. When re-examined the following days, where azotaemia stayed the same, however SDMA dropped from 18 µg/dL to 9 µg/dL. “Chivers” was also bright and alert, being then discharged after three days spent in hospital. “Chivers” had three more visits to the hospital before the end of the traineeship, with blood tests only being made in one of these occasions, with still slight azotaemia present and SDMA at 15 µg/dL.

1.3. Discussion

This was not the “typical” CKD case, as usually there is no definitive known cause for the disease in most animals. However, it was possible to determine that the most likely cause of kidney disease was due to an AKI caused by a prolonged exposure of anaesthesia which led to hypotension during surgery. It is worth knowing that, even though this was postulated as one of the possible causes, it is a rare occurrence, as usually blood pressure is constantly monitored in these procedures. Other differentials for AKI are nephrotoxicity due to NSAIDs, especially as they might cause a kidney insult when renal blood flow is affected, and age. By the time “Chivers” had

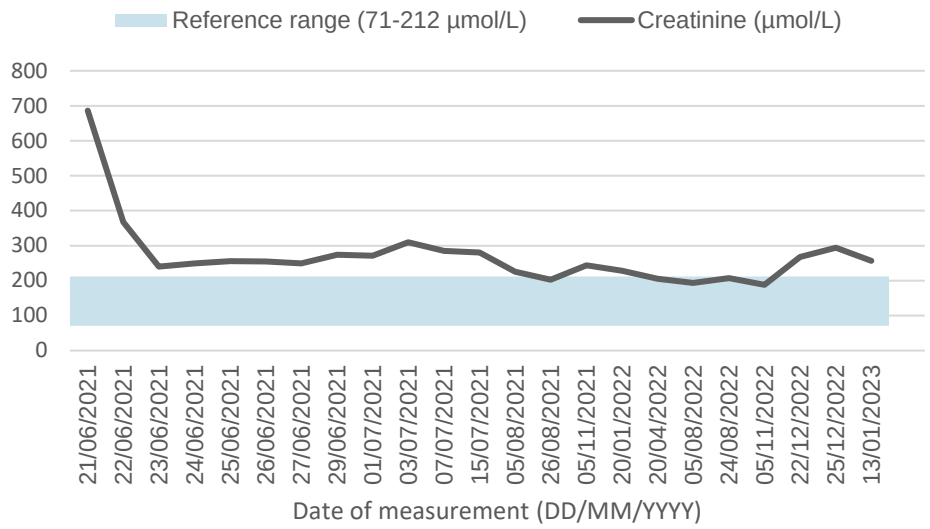
his surgery, he was already considered a senior cat, being that he was more predisposed to kidney damage under these circumstances (Jarnberg, 1998; Domi *et al.*, 2016; Dickerson *et al.*, 2017).

Other diseases that would cause anuria were also excluded before achieving the AKI diagnosis. For instance, obstruction of the lower urinary tract was excluded as bladder could be emptied by manual compression and urinalysis did not reveal any changes, besides increased UPC for the first week of fluid therapy (Goyal *et al.*, 2023).

Once the diagnosis of AKI was made, and due to the severity of azotaemia and clinical signs shown, it was advised the immediate hospitalization of the patient, so that intravenous fluid therapy could be started at a dose of 5 mg/kg/hr. This proved to be effective in improving the patient's condition, improving azotaemia and lowering SDMA values, as well as the patient becoming more alert and starting to graze the food and micturating. According to IRIS, the AKI grade initially identified in "Chivers" was grade IV, as creatinine values were 686 µmol/L. This, including the fact that the patient was previously anuric, meant that there was an emergency in applying treatment, as there is an increased risk of complications due to the azotaemia and functional renal failure. At this time, prognosis was not favourable, as IRIS AKI grade IV patients may die within five to ten days despite adequate supportive therapy (Cowgill, 2016). However, the patient survived and developed CKD as a consequence of the renal damage.

Slight hypercalcaemia was seen when the patient, which could either be associated with the disease itself, as the calcium status is usually altered in these patients, dehydration or it could be due to the fluid therapy, as ringers' lactate was used. Furthermore, hypercalcaemia resolved after reducing the fluid therapy's rate. No other changes in electrolytes were found throughout the case (Odunayo, 2014; Finch, 2016) .

Graphics 2, 3 and 4 show the different measurements done to assess the renal biomarkers. Once values started to stabilize near the reference range the measurements became more spaced (around every three months).

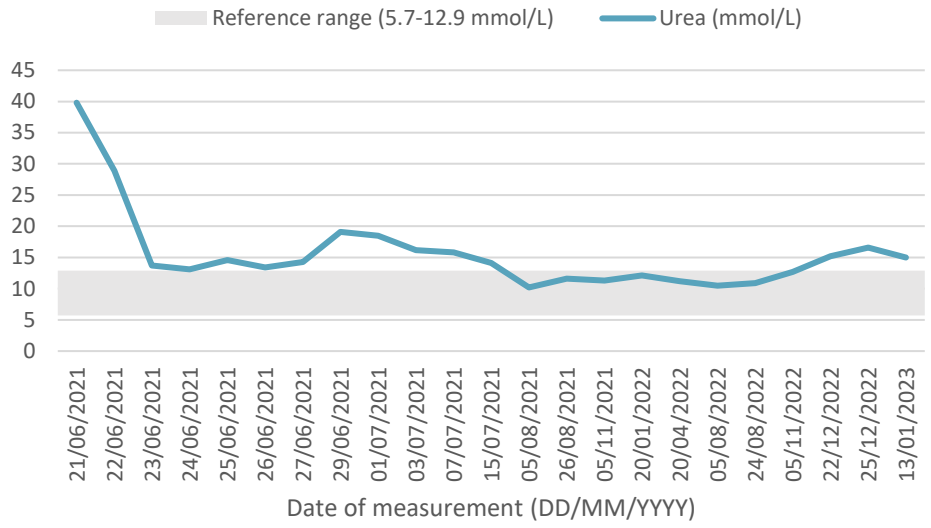


Graphic 2 - The evolution of "Chivers" creatinine levels throughout the treatment

Creatinine figures did not vary much from August 2021 onwards, besides up to December 2022, which indicates that the disease is under control. Urea and SDMA behaved similarly, being that the latter had an earlier peak (April 2022) than the other. However, SDMA was not constantly measured like the previous two, meaning that there could have been more fluctuation, or not, of this marker.

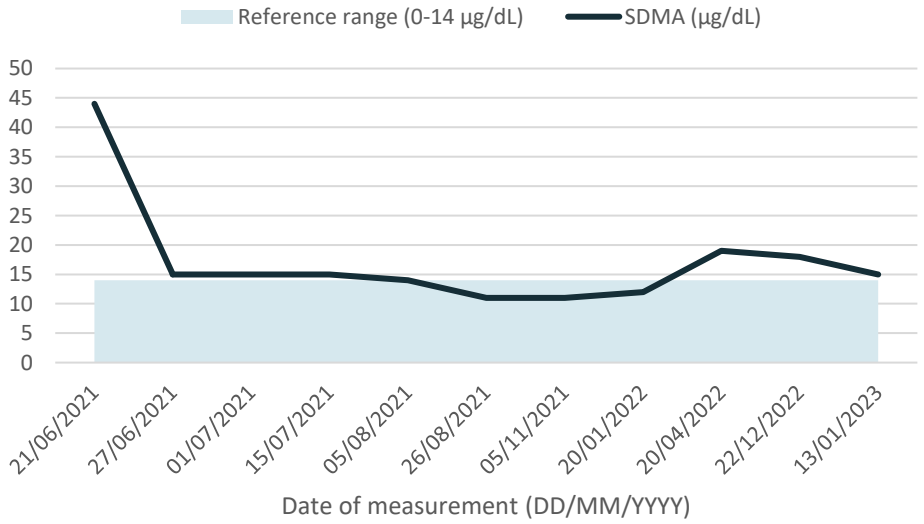
After 10 days of fluid therapy and management of clinical signs, the patient's diagnosis was updated to CKD, as no changes happened in renal parameters (persistent renal azotaemia). That being said, no changes were ever recorded in electrolyte levels, as treatment started right at the beginning of the disease, limiting the amount of phosphorus by changing the diet, and giving benazepril to prevent increases in blood pressure. Benazepril is also useful to reduce proteinuria, which also contributed to the success of the treatment, as no moderate or severe proteinuria ever emerged (Sent *et al.*, 2015).

"Chivers" had creatine values that fluctuated between 275 µmol/L and 188 µmol/L, putting him on stage II to III CKD. However, SDMA values were consistently under 15 µg/dL, except in two situations where it reached 18 µg/dL and 19 µg/dL, which would classify it at stage I CKD. As of today, when there are discrepancies between the two, SDMA should be the predominant marker for staging, as it is more sensitive and is less impacted by loss of lean body mass. Therefore, "Chivers" is a CKD stage I patient (*IRIS Kidney - Guidelines - IRIS Staging of CKD*, 2023).



Graphic 3 - The evolution of "Chivers" urea levels throughout the treatment

Hypertension was only noted in one situation in December, when “Chivers” was admitted again to hospital care after his “flare-up”. Blood pressure measurements revealed pressure of 180/122 (systolic/diastolic), which normalised two days after. There can be various explanations, as the hypertension may be a consequence of the CKD and deterioration of kidney function, or it can be due to stress, as “Chivers” needed to be re-hospitalized at that time (Lawson & Jepson, 2021).



Graphic 4 - The evolution of "Chivers" SDMA levels throughout the treatment

CKD is a progressive disease, it can be slowed down, but it is not possible to reverse the damage that is already present due to the fibrotic nature of the disease. Even though the disease was kept under check at almost all times, fluctuation in the renal values can be seen, and some complications showing up like vomiting, diarrhoea and inappetence. A good example of this was

observed from August 2022 to December 2022, where the renal scores were all within the reference range and no detectable change was detected. However, in December, “Chivers” has some episodes of vomiting and inappetence, where it was possible to identify, once again, a mild azotaemia. It is still uncertain whether the vomiting caused the azotaemia or the other way around, but it comes to show that even when the condition is controlled, kidney function might still worsen and there might be some flare-ups or episodes of deterioration. Other possible causes for vomiting are gastritis or inflammatory bowel syndrome, which are disease that have been associated with CKD previously. Maropitant was used at a dose of 1 mg/kg to help relief this clinical sign, and mirtazapine was also applied to help with nausea and food intake. The flare-up in December could also be a consequence of the vomiting, as dehydration due to it may have worsened azotaemia (pre-renal azotaemia). Other possible causes for this are UTIs or obstructions. However, there was no evidence found that would support the presence of neither of these.

This case evolution is a demonstrative frame of the importance of early detection and treatment of CKD as the disease progression was possible to halt at a very early stage, allowing to keep under control azotaemia and all clinical signs and complications associated. Another factor that heavily influences the treatment success is the owner’s attitude. Since the start the owner diligently monitored the cat, recording food and water intakes, and, in case of any changes detected, entered into contact with the veterinarians at Donaldson’s for guidance.

The prognosis for “Chivers” is good, as the disease was identified early, and it currently remains stable. Surveillance needs to be maintained at all times and any changes in water and food intake should be recorded and transmitted to the veterinary, so preventative measures can be applied before any more flare-ups happen. Prognosis is better in patients where CKD is diagnosed in earlier stages, as CKD progresses slowly in felines, as long as proper management is applied, which in turn will improve their quality-of-life (Boyd *et al.*, 2008). As “Chivers” was put on a renal diet early and with the hard work and attention the owner is providing the pet, it is expected that “Chivers” will have a mostly controlled disease throughout he’s life.

2. Patient “Molly”

“Molly” was an indoor cat with 14 years of age, neutered female and with vaccination and deworming up to date. She presented at Donaldson’s Vets in November 2022 with anorexia and weight loss.

2.1. Previous clinical history

“Molly” had a previous record of gingivitis and inflammatory bowel disease, with multiple flare-ups since they were diagnosed, in 2012. However, every episode of either of the diseases was promptly managed. In October 2022, “Molly” was admitted to the practice with anorexia and weight loss. No other changes were noted by the owner. Once admitted, blood sampling was

made, and a thorough clinical exam revealed only gingivitis. Once the blood work results came in, the diagnosis of CKD was made, as high urea and creatinine were detected. Glucose was also elevated in this case.

2.2. Managing and monitoring CKD

“Molly” was admitted with urea levels of 16.5 mmol/L, creatinine levels of 204 µmol/L and glucose levels of 8.1 mmol/L. The patient was put on fluids at a rate of 5 mL/kg/h for the day and sent home. The owner reported that the patient was doing well, besides some episodes of anorexia, and was put on a renal diet. As the disease was discovered in an earlier stage (in this case stage II), monitoring was done through visits every six months, being that no other changes appeared in the patient. SDMA was never measured, as the tutor was conservative about costs.

In the monitoring consults, blood pressure, UPC and renal values were measured. Blood tests done seven days after the initial diagnosis and in March 2023. The first revealed 16.7 mmol/L of urea and 209 µmol/L creatinine, which further cemented the CKD diagnosis. The later revealed similar levels of azotaemia, with 16.1 mmol/L of urea and 203 µmol/L creatinine, indicating that the disease seemed under control, besides some anorexia episodes, which were later attributed to IBD. UPC values were measured three times with recorded values of 0.17 in October and 0.20 in January and 0.20 in March (reference range from 0.00 to 0.40). Blood pressure was only measured once in a routine consult in November, with values within the reference range recorded in that instance. Treatment proved complicated has IBD made it hard to transition between diets, being necessary the addition of prednisolone to help maintain the renal diet.

2.3. Discussion

Unlike the previous case, “Molly” did not make any SDMA measurements, which could lead to some degree of uncertainty regarding the disease staging, as SDMA is a preferential marker when comparing with creatinine, because it suffers no influence from muscle mass and other external factors to the kidneys. Additionally, due to the owner’s reduced financial power, a much more conservative approach to the disease was made in comparison to the first case.

UPC and blood pressure were both accessed in the time in-between the renal value analysis. No proteinuria nor hypertension was detectable in the patient throughout the first six months of assessment, therefore not being necessary the use of ACEI’s.

As only three measurements were made (i.e., two measurements separated by seven days at the time of diagnosis and one measurement six months later) it was more difficult to monitor the evolution of the disease. However, due to the measurement’s similarity, it is possible to assume that the disease remained under control. According to the owner, no complications of the disease were detected during the first six months of monitoring, being that only a few instances of anorexia were reported. This was most likely due to the IBD and not related to the azotaemia. However, it was not possible to be sure as the azotaemia was not measured at the

time. IBD's clinical signs appeared once the new diet was introduced and once again in the beginning of January, being the disorder then treated with prednisolone, as it is a drug with proven effectiveness against it in patients with relapses at a dose of prednisolone of 5 mg (half a tablet) every other day. Gradual dose reduction should be performed until patient is again asymptomatic.

IBD can be triggered either by an exacerbated (i.e., allergic) reaction to one of the diet's ingredients *per se*. However, it is also possible to note a reaction due to differences in fibre and fat levels, fibre types and digestibility. Once prednisolone treatment started IBD was kept under control, with symptoms only appearing when the patient was weaned off the medicine. Hence, the patient was maintained with a low dose, given in alternate days. The use of prebiotics and probiotics could also be recommended to help control IBD, also possessing a positive effect in CKD patients, due to the relation between the gut microbiome and the progression of kidney disease (Trepanier, 2009; Malewska *et al.*, 2011; Corica & Romano, 2016; Hall, Jewell & Ephraim, 2022).

The prognosis for "Molly" was good, as the disease was diagnosed in an early stage of the disease (stage II), and the renal values remained somewhat the same throughout the first six months of monitoring, which could mean the disease is stable. Just like with "Chivers", the food and water intake should be monitored, and any changes in appetite should be recorded and transmitted to the veterinarian, so preventative measures can be applied. Because of the IBD, extra care should be made in order not to put "Molly" under stress, and changes in diet should also be avoided, so that clinical signs of the disease do not show up. A relapse in IBD could mean a reduction in body score condition, which would result in decline in quality of life, which would worsen her prognosis.

V. Conclusion

The curricular traineeship at Donaldson's Vets allowed the trainee to put in practice and consolidate the clinical knowledge obtained in the University of Évora. The chance to follow and cooperate with the professionals at Donaldson´s, together with the diversity of clinical cases that arrive on a day-to-day basis to the practice allowed for a personal and professional enrichment, which is sure help the trainee in his future.

CKD is considered one of the most common diseases and acknowledged as a multifactorial disease, with multiple possible causes. It is important to know that, independently of the suspected cause, action should be taken quickly when diagnosis is achieved, with the treatment aimed at correcting blood volume, and consequently azotaemia, and correcting electrolyte unbalances that may be occur. If known, the cause of CKD should be addressed, before advancing to dietary maintenance of CKD.

The owner's perception of the disease and a good relationship with the clinician is essential to achieve therapeutic success, as most of the "work" is done at home, with both correct dietary management and by monitoring food and water intake. Any symptomatic diseases associated with CKD should be managed, like nausea and vomiting through the use of

antiemetics, inappetence through the use of appetite stimulants and hypertension and proteinuria though the use of ACEI, as these may affect quality of life and promote disease progression.

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