UNIVERSIDADE DE LISBOA

Faculdade de Medicina Veterinária

CUTANEOUS AND RENAL GLOMERULAR VASCULOPATHY: A REVIEW OF CASES SEEN AT AN EMERGENCY VETERINARY PRACTICES IN UK

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Para a minha mãe

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Resumo

VASCULOPATIA CUTÂNEA E GLOMERULAR RENAL: UMA REVISÃO DE CASOS EM CLÍNICAS DE EMERGÊNCIA VETERINÁRIA NO REINO UNIDO

A vasculopatia cutânea e glomerular renal (CRGV), mais conhecida por *Alabama Rot*, é uma doença que foi descrita pela primeira vez em galgos de corrida em 1988, mas reconhecida desde 1995 nos Estados Unidos da América. No Reino Unido, a sua ocorrência tem aumentado desde 2012. Esta doença manifesta-se sobretudo por eritema e edema das extremidades, progredindo rapidamente para úlceras cutâneas, trombocitopénia e insuficiência renal aguda (IRA). Quando o quadro de insuficiência renal aguda se instala, geralmente é fatal. A causa desta doença ainda não é conhecida. A principal alteração histopatológica renal que confirma CRGV é a Microangiopatia trombótica (TMA), descrita nos humanos e cães. Atualmente, é desconhecido se a CRGV é uma nova doença da espécie canina ou se é uma modificação da síndrome hemolítica urémica, síndrome atípica hemolítica urémica, púrpura trombótica trombocitopénica e coagulação intravascular disseminada, que são as microangiopatias descritas nos humanos. Este estudo tem como objetivo sistematizar casos de cães com suspeita de CRGV avaliando se as lesões cutâneas se correlacionam com o aparecimento de IRA e se esta está associada a um pior prognóstico da doença. Foi realizado um estudo retrospetivo que consistiu na análise de 40 casos consultados em 26 clínicas com uma unidade de cuidados intensivos analisando a anamnese, sinais clínicos, exames complementares de diagnóstico, tratamento e prognóstico. Vinte e sete cães exibiam apenas lesões de pele e 13 lesões de pele e IRA. As lesões mais comuns encontradas foram abrasões superficiais e úlceras cutâneas, sendo estas mais prevalentes no grupo com IRA, presença de inflamação e dermatite, variando no tamanho. Foram observados alopécia, eritema e edema, em especial quando localizados nos membros e dígitos. Lesões com dimensão igual ou superior a 5 cm estão significativamente correlacionadas com o aparecimento de IRA ($p=0.029$). A mediana do tempo decorrido entre o aparecimento das lesões e o diagnóstico de IRA foi de 3 dias ± 5 dias. Além da azotémia, os cães com IRA apresentaram anemia, proteinúria, hematúria, hipostenúria, hipocalcémia, trombocitopénia, neutrofilia, enzimas hepáticas elevadas e hiperbilirrubinémia. Cinco cães foram submetidos a eutanásia (38,5%), devido a azotémia, não-resposta á fluidoterapia e oligoanúria, sendo que os restantes sobreviveram. Este estudo revela que os cães com azotémia ($p=0.001$), oligoanúria ($p<0.001$), hipocalcémia ($p=0.003$) e hipofosfatémia ($p<0.001$) estão associados a um prognóstico reservado. Contudo, o tratamento médico intensivo é indicado nestes casos, uma vez que existem resultados positivos com recuperação de IRA completa, como analisado em sete cães (53.8%) neste estudo. **Palavras-chave**: CRGV, *Alabama rot*, insuficiência renal aguda, lesão cutânea, microangiopatia trombótica

Abstract

CUTANEOUS AND RENAL GLOMERULAR VASCULOPATHY: A REVIEW OF CASES SEEN AT AN EMERGENCY VETERINARY PRACTICES IN UK

Cutaneous and renal glomerular vasculopathy (CRGV), more commonly known as *Alabama rot* is a disease first reported in racing greyhounds in 1988 but recognised since 1995 in the USA, and with increasing occurrence, since 2012, in the UK. This disease is characterised with acute erythema and oedema progressing rapidly to cutaneous ulcers of the extremities, thrombocytopenia and clinically relevant acute renal failure (AKI). When acute renal failure develops it is usually fatal. The cause of this cutaneous and renal glomerular vasculopathy is not yet known. Thrombotic microangiopathy (TMA) is the main renal histopathological change that confirms CRGV and has been described in humans and dogs. It is currently undefined if CRGV is a new canine disease or if it is a variation of the haemolytic uremic syndrome, atypical haemolytic uremic syndrome, thrombotic thrombocytopenic purpura or disseminated intravascular coagulation which are the TMA's reported in humans. The objectives of this present study are to review cases of dogs suspected with CRGV evaluating if the cutaneous lesions correlate with the developing of AKI and if this is associated with a worse outcome of CRGV. 40 cases from 26 first opinion emergency providers were analysed and their history, clinical signs, clinicopathological findings, diagnostics, treatment plan, and outcome observed. 27 dogs presented with only skin lesions and 13 with skin lesions and AKI. The most common macroscopic aspects of the skin lesions were superficial abrasions and cutaneous ulcers, particularly in the group of dogs with AKI, presence of inflammation and dermatitis, characterized by different sizes. Alopecia, erythema, and oedema were also observed, mainly when located in the limbs and digits. Lesions wider than five centimetres were significantly correlated with development of AKI ($p=0.029$). The median time between the presence of skin lesions and the diagnosis of AKI was 3 ± 5 days. Besides the azotaemia, dogs with AKI presented with anaemia, proteinuria, haematuria, hyposthenuria, hypocalcaemia, thrombocytopenia and neutrophilia, high serum liver enzyme activity, and hyperbilirubinaemia. Five animals were submitted to euthanasia (38.5%), due to azotaemia, no response to intravenous fluid therapy and oligoanuria. The remain survived. This study reveals that having azotaemia (0.001), oligoanuria ($p<0.001$), hypocalcaemia ($p=0.003$) and hypophosphatemia ($p<0.001$) was significantly correlated with a worse outcome. Nevertheless, intensive medical therapy is designated in these patients because successful outcomes with full recovery from AKI have been achieved as observed in seven dogs (53.8%) in this study.

Keywords: CRGV, Alabama rot, acute kidney injury, skin lesion, thrombotic microangiopathy

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ACEi – Angiotensin-converting-enzyme inhibitor

ADAMTS13 - a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13

AFAST – Abdominal Focused Assessment with Sonography for Trauma

aHUS – Atypical haemolytic uraemic syndrome

AKI – Acute renal injury

ALKP – Alkaline phosphatase

ALT – Alanine transaminase

aPTT - Activated partial thromboplastin time

ARBs – Angiotensin II receptor blockers

AST – Aspartate transaminase or aspartate aminotransferase

C3 - Complement component 3

CCB – Calcium channel blockers

CFB - Complement factor B

CFH - Complement factor H

CFI - Complement factor I

CK – Creatine kinase

CKD – Chronic kidney disease

CRGV – Cutaneous and renal glomerular vasculopathy

D+HUS – Diarrheal HUS

D-HUS – Non-Diarrheal HUS

DIC - Disseminated intravascular coagulation

EPOC® – epoc Veterinary Blood Gas, Electrolyte and Critical Care Analyser

FISH – Fluorescence in situ hybridization

GB – Great Britain

HUS - Haemolytic-uremic syndrome (HUS)

i.m. – Intramuscular

i.v. – Intravenous

INR – International normalized ratio

IQR – Interquartile range

IRIS – International Renal Interest Society

KC – Kennel Club

LDH – Lactate dehydrogenase

MAT - Microscopic agglutination test

MDB – Minimum Data Base

NSAIDs - Nonsteroidal anti-inflammatory drug

OR – Odds Ratio

PCR – Polymerase chain reaction

PEX– Plasma Exchange

PO – *Per os*

PT – Prothrombin time

RAAS – Renin–angiotensin–aldosterone system

s.d. – standard deviation

STEC-HUS – *E. coli* serotype O157:H7 Shiga toxin-producing associated with haemolytic uremic syndrome

Stx-HUS – *Shigella dysenteriae* shiga toxin-producing associated with haemolytic uremic syndrome

TFAST – Thoracic Focused Assessment with Sonography for Trauma

TMA – Thrombotic microangiopathy

TTP - Thrombotic thrombocytopenic purpura (TTP)

UK – United Kingdom

UOP – Urine output

UPC – Urine Protein Creatinine Ratio

US – Ultrasonography

USG – Urine Specific Gravity

Traineeship report

As part of the Integrated Master's Degree in Veterinary Medicine from the Faculty of Veterinary Medicine of the University of Lisbon, I completed a six month internship in a 'VetsNow' clinic based in Stoke-on-Trent, Staffordshire, United Kingdom (UK). I fulfilled an estimate of 1700 hours in a high-quality provider of pet emergency care, working out-of-hours only, nights during the week, weekend and bank holidays.

My shifts started at six pm and finished at nine am, lasting 15 hours. During this time, we had a large case load, receiving patients from the more than 20 general practices around Staffordshire.

As I worked in emergency and critical care, I participated in different services throughout the night. These services were internal medicine where I often performed treatment plans for the patients solving clinical cases, treatment and monitoring the inpatients overnight and improved my skills in practical procedures such as placement of catheters, drug administration, blood sampling, blood transfusion, urinary catheterization, clinical examination, and consultation skills. I frequently triaged the patients independently or over the phone, deciding if the animals needed emergency care immediately.

I did also work in the surgery department where I regularly assisted all the emergency surgeries, being most commonly foreign bodies, caesareans, gastric dilation-volvulus, pyometras, amongst others. In this department, I was able to improve my knowledge in anaesthesia as I practiced procedures such as choosing and performing the most suitable pre-medication, induction, intubation and general anaesthesia for each patient.

In the diagnostics department I learned how to perform and to interpret emergency diagnostics such as EPOC, minimum data base (glucose, packed cell volume, total solids, lactate and urea), AFAST and TFAST (Focused Assessment with Sonography for Trauma, for abdomen and thorax, respectively), X-ray (positioning patients, using sedation when necessary and read the results), endoscopy and analyse blood and urine smears.

Every month, I had to prepare a Power Point presentation for my mentor with cases that I had assisted, including history, clinical exam, differential diagnostics, diagnostics, procedures performed, treatment and outcome. At the end of the presentation, I then discussed, in details, the cases that I presented.

During this period, I had the opportunity to integrate into the team that worked during the day at Lime Trees Veterinary hospital. I worked in general practice and a referral centre in small animal surgery and internal medicine, where I had the opportunity to assist surgeries such as pyometras, dog and cat castrations and ovariohysterectomies. I also performed independently with the supervision of a senior vet, a cat ovariohysterectomy using the flank technique. I then assisted the internal medicine referral consults with Doctor Hywel Parry (BVM&S CertSAM MRCVS RCVS).

On my first week of the traineeship, I prepared a case that I assisted during my traineeship about Addison's disease and was selected to present it to a large audience and a panel of judges along with other six students and trainees, in the VetsNow Annual congress on the 8th of November 2018 in Harrogate, UK. During the congress, I had the opportunity to meet other qualified vets and assist the lectures about ECC.

I. Introduction

1. Cutaneous and renal glomerular vasculopathy

Cutaneous and renal glomerular vasculopathy (CRGV) is a disease firstly reported in racing greyhounds in 1988. It was recognised since 1995 in the USA (Carpenter, Andelman, Moore, & King, 1988), and with increasing occurrence, since 2012, in the UK (Holm, Hawkins, Jepson, Robin, Newton, Stanzani, McMahon, Cianciolo, Pesavento, Carr, Cogan, Couto, & Walker, 2015). This disease usually manifests itself with acute erythema and oedema progressing rapidly to cutaneous ulcers of the extremities, thrombocytopenia and clinically relevant acute renal failure (AKI). When acute renal failure develops it is usually fatal (Hertzke, Cowan, Schoning, & Fenwick, 1995). The cause of this cutaneous and renal glomerular vasculopathy is not yet known (Carpenter, et. al, 1988; Skulberg, Cortellini, Jepson, Chan, & Stanzani, 2018) however, the chronology of presentation, diagnostic testing and small sample size are limiting factors (Jepson, Cardwell, Cortellini, Holm, Stevens & Walker, 2019).

Terrestrial Animal Health Code of the World Organisation for Animal Health (OIE) defined *emerging disease* as a

> new occurrence in an animal of a disease, infection or infestation, causing a significant impact on animal or public health resulting from: a) a change of a known pathogenic agent or its spread to a new geographic area or species; or b) a previously unrecognised pathogenic agent or disease diagnosed for the first time (Moutou & Pastoret, 2015, p.41).

The outbreak pattern of CRGV in the UK accords with the definition of a newly emerging disease as no cases were reported prior to 2012. However, although this indicates the disease was not utterly known, it may not have been the case. It may simply not have been recognised owing to a very low incidence in the population prior to 2012 (Stevens, Jepson, Holm, Walker, & Cardwell, 2018). Up to this date, there are more than 180 confirmed cases since 2012, when CRGV was firstly reported in the UK (Anderson Veterinary Specialists), with only ten cases confirmed in 2019 (January until March). This contrasts with the year 2018 whereby the end of March 34 CRGV cases was confirmed. The leader investigators on CRGV in the UK (David Walker from Anderson Moors Veterinary Specialists), attributes this event to the different climate changes from January to March in 2018, compared to the year 2019, but currently, this has been the object of on-going research (Walker, D. personal communication, 2019).

In 1988, Carpenter firstly characterized this disease syndrome which he observed only in racing training greyhounds since 1985 (n=168). These dogs manifested multifocal cutaneous

erythema, ulceration, and distal limb oedema. This syndrome was named '*Alabama Rot*' or '*Greenetrack disease*', as many cases were reported and affected many dogs in Greenetrack Racing Park in Alabama. Although this disease is commonly known by '*Alabama Rot*' (Carpenter, et. al, 1988) or '*New Forest disease*' (Hertzke, et. al, 1995) both diseases should more reasonably be entitled cutaneous and renal glomerular vasculopathy (CRGV) based on their histopathological features, rather than their geographic distribution (Cortellini & Humm, 2015).

It was thought this disease only affected greyhounds as this breed hold their own exclusive physiologic adaptations, owed to the fact they were racing sighthounds. As an example, these dogs have more muscular mass, higher haematocrit, elongated carpal, tarsal, metacarpal, and metatarsal bones and an improved sense of sight. All these features probably contributed to the unique hematologic and biochemical characteristics of Greyhounds compared with non-Greyhound breeds (Zaldívar-López et al., 2011). When the outbreak, however, occurred in the UK, CRGV was reported in a variety of breeds, including, the English springer spaniel, flat-coated retriever, whippet, border collie, Jack Russell terrier, Doberman, Labrador retriever, cocker spaniel, Staffordshire bull terrier, Hungarian vizsla, Weimaraner, Dalmatian, Tibetan terrier and crossbreds (Holm et al., 2015), with only one greyhound reported in the UK (Hendricks, 2000) and a Great Dane in Germany (Rotermund, Peters, Hewicker-Trautwein, & Nolte, 2002). Only two greyhounds have been affected with CRGV (with AKI) in the UK, therefore, it is still uncertain whether CRGV and 'Alabama rot' are identical or separate disease entities (Holm & Walker, 2018).

This disease presents itself in four different distinct manifestations (Carpenter, et. al, 1988): dogs can only manifest skin lesions without showing any signs of systemic illness. To this date, there are no studies that reveal how many dogs in the UK have CRGV with skin lesions only (Holm et al., 2015). The second course was seen in dogs with typical skin lesions, systemic illness signs and a quick onset of azotaemia; third manifestation is described as a cutaneous ulceration clinically normal with renal failure within ten days of onset of skin lesions; the fourth manifestation was identified in seven dogs that developed azotaemia before cutaneous ulceration (Carpenter, et. al, 1988). It is important to notice that the presence of skin lesions along with the presence of AKI due to other causes, is rarely reported in dogs (Jepson et al., 2019).

2. Aetiology and Differential Diagnosis

After CRGV been recognized in the UK, investigations were carried out to recognise whether the disease was attributable to any previously known causes of canine AKI, or whether the aetiology was analogous to human haemolytic uremic syndrome (HUS), atypical haemolytic uremic syndrome (aHUS) or thrombotic thrombocytopenic syndrome (TTP). No single unifying cause was able to be identified from these results (Holm et al., 2015).

When AKI is confirmed through laboratory analyses, the first method to approach is to rule out any known causes, before assuming CRGV is present. Chronic kidney disease (CKD) and post-renal causes should be excluded first. To assess post-renal causes abdominal imaging is helpful. This can be useful to evaluate renal architecture, which combined with clinical history can exclude CKD. Once these two illnesses been excluded, other potential causes of AKI and pre-renal causes must be considered (Holm et al., 2015)

Pre-renal causes are excluded through urine specific gravity, lack of response to intravenous fluids and exclusion of hypoadrenocorticism (basal cortisol blood test and ACTH stimulation test). A detailed clinical history regarding potential nephrotoxic substances ingestion should be obtained, full urinalysis including culture should be performed, leptospirosis testing (PCR on blood/urine before antibiotic therapy and microscopic agglutination test), abdominal imaging to assess renal architecture, analysis of serum electrolyte concentrations should all be considered (Holm & Walker, 2018).

When these patients present themselves to their primary veterinary practice with skin lesions, it is a common procedure to administrate nonsteroidal anti-inflammatory drugs (NSAIDs) in the management of these wounds (Holm et al., 2015) which are known to have indirect negative effects in the kidneys (Lomas & Grauer, 2015). Holm et al. (2015), stated that eleven of the dogs (36.7 per cent) received NSAIDs previously to the diagnosis of AKI. It is possible that their use aggravated AKI, but the histopathologic lesions were not consistent with NSAIDs being the particular cause of the AKI in these dogs (Holm et al., 2015).

In the largest case series of dogs with CRGV to date (Holm et al., 2015), many possible pathogens that can be the potential aetiology of CRGV were considered. One possible condition investigated was Leptospirosis. The predominant clinical signs of acute leptospirosis relate to the presence of acute kidney injury and liver damage (Schuller et al., 2015), which are similar to CRGV findings. Renal histopathology in dogs with leptospirosis (no typical hepatic lesions were identified) is not compatible with CRGV. Five dogs had positive titers, although at a low concentration (1:100–1:800). Nevertheless, all these dogs had been vaccinated less than one year before testing. Moreover, although it is discussable, only single titers greater than 1:1600 are considered relevant for indicating an infection in vaccinated dogs (Miotto et al., 2018;

Schuller et al., 2015).

In this same study, faecal culture was executed, and *Escherichia coli* was identified. Nevertheless, multiplex polymerase chain reactions (PCRs) for *E. coli* virulence genes were negative. Shiga toxin has not been identified in dogs with HUS before (Holloway, Senior, Roth, & Tisher, 1993).

A viral ethiopathogeny was hypothesized as well in this study and viral metagenomics and canine circovirus PCR were performed, although all results were negative, and histopathologically there was no evidence of viral cytopathic effect (cytoplasmic inclusion bodies) in any of the tissues examined. (Holm et al., 2015). Nevertheless, negative results for viral metagenomics do not eliminate a viral cause as these results could indicate that virus was present in low copy numbers, or that the virus was too weakly related to known viruses used for sequence alignment, or that the sample used was too autolyzed to preserve the virus. (Jepson et al., 2019)

Numerous other causes were deliberated in this UK case series such as *Borrelia* (PCR and serology were negative), and renal heavy metal concentrations (lead, arsenic, and cadmium; concentration below reported reference intervals) (Holm et al., 2015).

Macdonald (2015), is conducting a research that hypotheses that the bacteria *Aeromonas hydrophila* can be the pathogen of CRGV. These bacteria can live in areas were some dogs normally walk and were confirmed with the disease. These areas feature substantial amounts of water (as result of an unusually high rainfall) and both the running water and the standing water were at 4°C for some weeks around the time of the cases. Only dogs appeared to be affected, with no registered mortalities in wild ponies, foxes, cattle or deer (Macdonald, 2015). Correspondingly, *A. hydrophila* in dogs can mimic Leptospirosis infections (Andre-Fontaine, Monfort, Buggin-Daubie, Filloneau, & Ganiere, 1995). Moreover, *A. hydrophila* is identified to cause ulcerative skin lesions in both ornamental and farmed fish, with consequent kidney failure. It is a very toxigenic organism and so the kidney failure will be a toxin response, making bacterial isolation from target tissues nearly impossible. Currently, studies are being conducted in order to confirm or not if this is the potential cause of CRGV (Macdonald, 2015).

3. Pathogenesis

Thrombotic microangiopathy (TMA) is the main renal histopathological change that confirms CRGV (Holm et al., 2015), and has been described in humans (Shatzel & Taylor, 2017) and dogs (Holloway et al., 1993). TMAs are characterised by inflammation and damage to the arteriolar endothelium, leading to platelet activation and aggregation and therefore to a widespread formation of microthrombi which constricts affected vessels. Erythrocytes suffer

shear injury and the resultant schistocytes are removed by the reticuloendothelial system, resulting in extravascular haemolysis. Intravascular haemolysis can similarly occur when the erythrocyte lesions are severe (Jepson et al., 2019). Fragmented red blood cells are probably cut as blood flows through turbulent extents of the microcirculation that are partially narrowed by microthrombi. This process leads to microangiopathic haemolytic anaemia (Moake, 2002). This acute haemolysis progresses to anaemia and the global formation of microthrombi outcomes in a consumptive thrombocytopenia. The microthrombi finally occlude the blood vessel lumen entirely, leading to reduced organ perfusion and death of the affected tissue (Holm & Walker, 2018) leading to an eventual multiorgan dysfunction (Hertzke et al., 1995).

Haemorrhage can occur when the platelet count is less than roughly 30-50 x 10^9/L. Classic laboratory reference ranges for platelet counts vary between 200–400 x 10^9/L (Hohenhaus & White, 2012).

Therefore, clinical manifestations of TMAs are thrombocytopenia, anaemia, fragmentation of erythrocytes (schistocytes), and particularly elevated serum levels of lactate dehydrogenase (LDH) (Moake, 2002). Haemolysis can be established over laboratory markers results, such as lactate dehydrogenase (LDH), haptoglobin, and bilirubin, which are all increased (Williams & Marques, 2016). Serum LDH level is increased due to the release of red blood cell LDH as a result of intravascular haemolysis (Cohen, Brecher, Bandarenko, Hill, & Carolina, 1998).

Glomerular TMA in CRGV is characterised microscopically by hyaline thrombi within capillaries and sometimes the afferent arterioles, segmental to global congestion and necrosis, and thickening of glomerular capillary walls, which is consistent with findings in humans histopathology in TMAs (Hertzke et al., 1995).

Two major differential diagnosis for TMAs reported to occur in domestic animals are, more commonly CRGV and haemolytic uraemic syndrome (HUS) (Carpenter, et.al 1988; L. Holm & Walker, 2018; Skulberg et al., 2018).

3.1 Thrombotic microangiopathies in humans

TMA, in humans, include principally two syndromes, thrombotic thrombocytopenic purpura (TTP) and haemolytic uremic syndrome (HUS). Each of these syndromes appears to be caused by numerous distinct pathophysiologic mechanisms (Zheng & Sadler, 2008).

It is currently indefinite if CRGV is a new canine disease or a variation of the haemolytic uremic syndrome (HUS), atypical haemolytic uremic syndrome (aHUS), thrombotic thrombocytopenic purpura (TTP) and disseminated intravascular coagulation (DIC) which are the TMA's reported in humans. The renal glomerular histopathologic findings resembling glomerular thrombosis, necrosis and haemorrhage are similar to the lesions found in CRGV (Carpenter, et. al, 1988).

3.1.1 Haemolytic uraemic syndrome

Haemolytic uraemic syndrome (HUS) is characterised by the presence of acute renal failure along with low platelet count and microangiopathic haemolytic anaemia (Zheng & Sadler, 2008).

Renal microcirculation becomes impaired due to the obstruction of small vessels from the fibrin thrombi that form in this particular area (Caprioli, Remuzzi, & Noris, 2011).

The term HUS was firstly reported by Gasser and colleagues in 1955. It was described as an acute haemolytic anaemia associated with kidney injuries in young children after an enteric or respiratory infection. HUS is categorized into three categories: HUS due to infections, often connected with diarrhoea (D+HUS), with the rare exception of HUS due to a severe disseminated infection caused by streptococcal organisms; HUS related to complement abnormalities or to factor ADAMTS13 deficiency, also recognised as 'atypical HUS' and is not diarrhoea associated (D-HUS); and HUS of unidentified aetiology that generally occurs in the course of systemic diseases or physiopathologic conditions. Examples of this are pregnancy, after transplantation or after drug assumption (Salvadori & Bertoni, 2013). Typical HUS (D+HUS) is associated with a prodromal diarrheal illness regularly caused by infection with *E. coli* O157:H7 (STEC-HUS) or a different Shiga-toxin-producing strain of bacteria (e.g. *Shigella dysenteriae*) (Stx- HUS). This type of HUS constitutes about 95% of the HUS cases in children, with the disease being rare to occur in adults (Zheng & Sadler, 2008). This Shiga-like verotoxins injure the endothelium, widely assumed to be the primary cause of renal dysfunction (Furlan & Lammle, 2001). Atypical HUS (aHUS) is quite sporadic and very heterogeneous as to its aetiology, age of onset and clinical presentations (Zheng & Sadler, 2008).

Infectious HUS, is related with prodromal diarrhoea followed by acute renal failure (with anuria), and considered a disease with a good outcome (Caprioli et al., 2011). This illness is confirmed by combining a positive stool test for Shiga-toxin-producing *E. coli*, a prodromal illness characterized by diarrhoea (often haemorrhagic) and the presence of TMA (Nalluru, Sridharan, Go, Said, & Marshall, 2018).

This type of infections requires prompt consideration of antibiotic treatment, before or after culture results are known. Nevertheless, such treatment may increase the risk of developing HUS (Freedman et al., 2016). Supportive treatment, such as isotonic volume replacement/ expansion, red blood cell, and platelet transfusion and, for severe AKI, haemo or peritoneal dialysis (Bitzan, 2009), yet plasma infusion or exchange are normally the chosen therapy to these patients (Schwartz et al., 2016).

Furlan's findings confirmed that a deficit of ADAMTS13 activity is not a feature of HUS (Furlan & Lammle, 2001). These conclusions are consistent with previous clinical studies that

have demonstrated that plasma exchange (PEX) is not indicated in this disorder, however conservative therapy with fast volume repletion and dialysis when needed provides the best survival advantage and first-line treatment for Diarrheal HUS (D+HUS) (Michael, Elliott, Craig, Ridley, & Hodson, 2009; Bitzan, 2009). Nonetheless, a small number of cases may actually have aHUS or TTP⁄HUS and necessitate plasma therapy urgently (Clark, 2012).

The microscopic and ultrastructural glomerular changes in patients affected with CRGV are extremely similar to lesions described in children with the classic form of HUS (Hertzke et al., 1995). Thickening of the glomerular capillary walls, inconstant in degree, caused by the deposition of an eosinophilic, faintly PAS-positive hyaline or granular material between the basement membrane and the endothelium, and hyaline or granular thrombi within glomerular capillaries and arterioles were the major histologic features (Vitsky, Suzuki, Strauss, & Churg, 1969). Even though HUS and CRGV have similar features, they contrast in some aspects. Cutaneous ulcerations are classically present in CRGV but are not related to HUS. Likewise, watery diarrhoea, followed by bloody diarrhoea, abdominal cramps along with vomiting and nausea marks the onset of HUS in children (Salvadori & Bertoni, 2013), but it is not seen in CRGV. Another feature that differs from HUS and CRGV is age. Whereas the CRGV appears in young adults, HUS is perceived in young children. (Hertzke et al., 1995; Holm & Walker, 2018). In domestic animals, HUS has been reported in calves (Valli & McSherry 1973), in a cow (Roby et al., 1987), four horses (Morris et al., 1987; Dickinson et al., 2008), three cats (Aronson & Gregory, 1999), three rabbits (García et al., 2002) and five dogs (Holloway et al., 1993; Chantrey, Chapman, & Patterson-Kane, 2002; Orco, Bertazzolo, Pagliaro, Roccabianca, & Comazzi, 2005).

Five to ten percent of all cases of HUS in humans are unrelated to infections by Shiga toxin-producing *E. coli* and are classified as atypical. aHUS is an ultra-rare disease regularly associated with progressive renal function deteriorating, microangiopathy disease with anaemia, thrombocytopenia and is characterized by a high mortality rate (Caprioli et al., 2011). It results of uninhibited activation of the alternative complement system due to genetic mutations impacting complement regulatory proteins such as complement factor H (CFH) and membrane cofactor protein (MCP), factor I and thrombomodulin (THBD) (Salvadori & Bertoni, 2013; Stevenson, Leung, & Winters, 2016). Immunosuppressive therapy should also be considered in patients with aHUS due to factor-H auto-antibodies (Fakhouri, Frémeaux-Bacchi, & Loirat, 2013). Cyclophosphamide pulses along with steroids administration and PEXs led to a prolonged decrease in CFH antibody titters and a favourable outcome in patients with aHUS (Boyer et al., 2010).

With this condition, it has been reported skin lesions alongside haemolysis and AKI, in contrast,

has not been described with STEC-HUS (Ardissino et al., 2014). Atypical HUS has not been reported in dogs, although, CRGV may have some similarity to this illness, particularly given the concurrent findings of skin lesions and AKI identified in both diseases. An infectious or environmental trigger for CRGV may be suspected, given the number of contact between dogs that developed skin lesions with or without AKI (Holm et al., 2015).

3.1.2 Thrombotic thrombocytopenic purpura

Thrombotic thrombocytopenic purpura (TTP) is an unusual hematologic disorder, characterized by microangiopathic haemolytic anaemia (MAHA) with schistocytes in the blood smear, thrombocytopenia, and variable stages of organ damage, including renal function impairment, neurological symptoms including stroke, and fever (Shatzel & Taylor, 2017). The aggregation of platelets in the microvascular system leads ischaemia in the brain, causing neurological signs, and other organs (Ruggenenti, Noris, & Remuzzi, 2001). Despite the fact that AKI is common on HUS, it is not a severe condition in TTP (Phillips, Westwood, Brocklebank, Marchbank, & Gale, 2016).

It has been described in both acquired and inherited deficiencies forms, in the activity of von Willebrand factor (vWF) cleaving protease (ADAMTS13) (Lewis & Meyers, 1996; Caprioli et al., 2011; Jepson et al., 2019). The acquired form is the most common and ensues when IgG autoantibodies bind and remove ADAMTS13 (Stevenson et al., 2016). Furlan, et. al (1996) and Tsai (1996), independently isolated and characterized this new metalloprotease from human plasma that cleavages vWF at the Y1605- M1606 bond of the subunit. This metalloprotease was identified in 1996 as the 13th member of the ADAMTS family (A Disintegrin And Metalloprotease with Thrombospondin type 1 repeats) (Fujikawa, Suzuki, Mcmullen, & Chung, 2001). ADAMTS13 is the cleaving protein of von Willebrand factor (vWF). vWF is crucial for primary haemostasis. It allows adhesion of circulating platelets and thrombi formation where the endothelium suffered an injury (Zheng & Sadler, 2008). Physiologically, vWF is degraded into smaller circulating forms by ADAMTS13 with the influence of shear stress (Stokol, 2008). In patients diagnosed with TTP, ADAMTS13 level is less than 5% (normal range: 60–123%) (Park, Waldrum, & Marques, 2010). The activity of this protease is deficient, increasing the accumulation of ultra large vWF multimers that are highly reactive with platelets. As a result, large, potentially occlusive platelet thrombi are formed (Moake, 2002).

According to the Guidelines on the Use of Therapeutic Apheresis in Clinical Practice plasma exchange (PEX) is the first-line treatment either as a primary standalone treatment or in conjunction with other modes of treatment (Category I) (Schwartz et al., 2016). PEX removes ADAMTS13 autoantibodies and unusually large multimers of von Willebrand factor besides

replenishment ADAMTS13 (Brocklebank, Wood, & Kavanagh, 2018). Using PEX as a therapy, 91 percent of these patients survive an episode of TTP (Bell, Braine, Ness, & Kickler, 1991); Schwartz et al., 2016).

Acquired TTP, commonly requires immunosuppression as the treatment as 80 percent of these patients enters remission with plasma therapy, however, one-third of them have a relapse. The relapse rate is greater amongst survivors with ADAMTS13 activity <10 percent (estimated risk for relapse at 7.5 years, 41 percent) than survivors with ADAMTS13 activity of 10 percent or more (Hovinga, Vesely, Terrell, Lammle, & George, 2010).

Rituximab, a monoclonal anti-CD20 antibody has been used efficaciously in patients with thrombotic thrombocytopenic purpura (Bresin et al., 2009) and anti–ADAMTS-13 autoantibodies refractory to standard therapies (Fakhouri et al., 2005).

Although TMA is a characteristic feature of both aHUS and TTP, they differ in terms of clinical manifestations. In aHUS, the lesions and clinical symptoms are mostly restricted to the kidneys, whereas the pathologic changes of TTP are more extensively distributed, more often with central nervous system signs (Salvadori & Bertoni, 2013).

3.1.3 Disseminated Intravascular Coagulation

DIC is an acquired syndrome defined by the intravascular systemic activation of coagulation pathways and excessive microthrombi formation locally and at areas distant from the site of the original injury in small and medium-sized vessels, and eventually organ dysfunction. Consumption and exhaustion of platelets and coagulation factors lead to bleeding and haemorrhage into tissues (Taylor, Toh, Hoots, Wada, & Levi, 2001).

DIC occurs as a complication of infections, solid cancers, hematologic malignancies, obstetric diseases, trauma, aneurysm, and liver diseases, amongst others, and each nature of DIC presents distinguishing features associated to the primary disorder (Wada et al., 2013).

TMA is similar to DIC (Skulberg et al., 2018), however clinical pathologic findings in dogs with CRGV do not suggest this condition. Coagulation profiles were within normal limits, and fibrin degradation products were negative (Hertzke et al.,1995) whereas in DIC prothrombin time, in 50 to 75 percent of cases, is prolonged and activated partial thromboplastin time (aPTT) is prolonged in 50–60 percent of patients with DIC, but a normal or shortened aPTT may also be seen (Bick, 1996). Fibrin-related markers, such as fibrinogen and fibrin degradation products are elevated (Prisco et al., 1989).

4. Epidemiology of CRGV

There are case reports from throughout the UK, but 36.7 percent of the cases come from The New Forest, Hampshire. This percentage can be explained by the locality of the investigators that conducted this study and the increased interest and awareness amid local veterinarians in that area (Holm et al., 2015). The North-east of England and the New Forest region of south England have the highest five-year density of CRGV cases (Stevens, et al., 2018).

A possible winter/spring seasonality is proposed, as the case incidence has been the highest these months (92 percent of cases are reported between November and May), yet there are cases all the rest of the months, but less in number (Stevens et al., 2018). During the colder months in the UK maximum temperatures are increasing and may have provided a favourable habitat for an evolving organism or a new ecological niche for a pathogen that had always been existing in the environment but was beforehand unable to flourish in the comparatively cooler conditions of previous decades (Stevens et al., 2018).

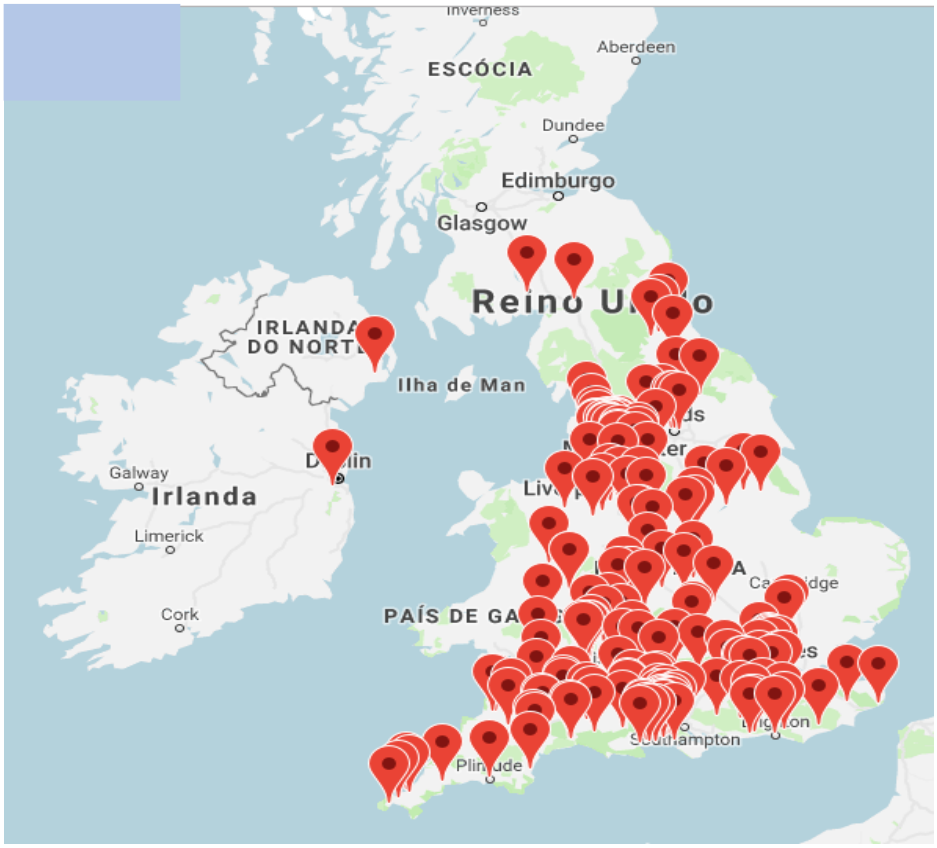
There has been a distribution of cases all over the UK, more specifically in 39 counties. Only a single case has been identified in Ireland (Figure 1) (Holm & Walker, 2018).

D+HUS is often sporadic, but reports of large outbreaks have been reported (Salvadori & Bertoni, 2013) which is similar to the epidemiological findings of CRGV (Stevens et al., 2018). Outbreaks of *E. coli* O157 have been reported in beef cattle and their products (Hussein, 2007) with cattle and sheep being the main reservoirs. The most important route of transmission is thought to be food contaminated with animal faeces (Ferguson et al., 2005), although in CRGV, pastures were the habitat least linked with CRGV occurrence. Combined with the decreasing domestic livestock densities, it suggests that it is unlikely CRGV is the result of a livestock-related pathogen to which dogs are exposed while walking across pastures, either from contact with the livestock themselves or their excretions (Stevens et al., 2018). This is, however, contrary to the findings in human HUS (Ferguson et al., 2005).

The incidence rate of D+HUS varies according to countries and climate and is higher in colder countries. For example, the incidence rate in Scotland (3.4×10^5 children under age 5) is higher than the overall incidence rate in Great Britain (1.54×10^5 children under age 5) (Lynn et al., 2005), which differs from CRGV, with only two cases reported in Scotland (Figure 1). Habitat, woodlands, and lowland dry heath communities were the variable identified to have the highest relative contribution to CRGV occurrence. The woodlands provide a rich habitat for a wide range of wildlife, plants, and fungi and this diversity makes it very difficult to isolate a single pathogen that might be the cause of CRGV (Stevens et al., 2018).

In conclusion, the uppermost relative probability of CRGV occurrence is associated with a range of agroecological factors more specifically, woodland and heath habitats, decreasing cattle and sheep densities, increasing maximum temperatures in winter and, to a lesser scale, spring, and autumn, and higher mean rainfall in winter and spring (Stevens et al., 2018).

Figure 1- Map showing the distribution of confirmed cases of cutaneous and renal glomerular vasculopathy (to the end of April 2019) (Adapted from https://www.vets4pets.com/stop-alabama-rot/).



Caption: The area from which cases have been reported covers much of the south and west of the UK, while the east appears to have a lower incidence of cases

5. Signalment

A previous case series conducted by Stevens et. al (2018), investigated the signalment risk factors for CRGV in UK dogs. It was the first and only study including 101 dogs diagnosed with CRGV showing that breed, kennel club (KC) breed group, and neuter status are significantly associated with a confirmed diagnosis of the disease, and that age group is not an important risk factor. Specific breeds have more probability of being a CRGV case compared with crossbreeds (80% of dogs in this study were purebreds and only 19 percent were crossbreds). Between the breeds more likely to be affected, there are specific ones with

increased odds ratio such as the flat-coated retriever (Odd Ratio (OR) = 84.48), Hungarian vizsla (OR = 40.98), Manchester terrier (OR = 41.41), Saluki (OR = 27.46), whippet (OR = 22.43), English springer spaniel (OR = 11.41) and bearded collie (OR= 10.85). Breeds with decreased odds included German shepherd dogs (OR = 0.45), Jack Russell terriers (OR= 0.37) and Staffordshire bull terriers (OR= 0.50). It remains mysterious whether this is due to a truly increased breed-associated risk, or due to the increased popularity of these breeds in areas experiencing a higher case incidence of CRGV (Stevens et al., 2018).

Gundogs[1] and hounds[2], two KC breed groups, are nine and ten times more likely to diagnosed with CRGV than terriers[3], according to this same study. Toy dogs[4] were not among the breeds affected by CRGV, therefore this group was excluded from the study (Stevens et al., 2018).

Female and neutered dogs are more likely to be diagnosed with CRGV, but the reasons behind these results are unclear. Nevertheless, previous reports have shown being female is a risk factor for HUS in human beings (George & Nester, 2014), although this does not appear to be the same in the other TMAs. Being neutered is also a risk factor with neutered dogs being 3.36 times more likely to be diagnosed with CRGV (Stevens et al., 2018).

The age range for dogs with CRGV is between 1.73 and 4.11 years old (Stevens et al., 2018), although in previous studies can range between 1.00 and 11.75 years (median 4.90 years) (Holm et al., 2015) and between10 months and 8 years, median 4 years old (Skulberg et al., 2018).

6. History and clinical signs

Clinical signs have acute onset before presentation to the first-opinion practice and the patients show a rapid clinical deterioration (Skulberg et al., 2018). Typically, skin lesions develop before AKI occurs. After 11 to 21 days of the presence of these skin lesions, AKI rarely occurs (Holm & Walker, 2018). Systemic signs develop a median of four days later (Holm et al., 2015), however, a proportion of dogs do not develop AKI, and only have lesions restricted to the skin, recovering from them uneventfully (Carpenter, et. al, 1988; Holm & Walker, 2018).

These injuries involve the distal extremities (Figure 2 and 3) (Carpenter, et. al, 1988; Holm &

[1] Gundogs – "Dogs that were originally trained to find live game and/or to retrieve game that had been shot and wounded. This group is divided into four categories - Retrievers, Spaniels, Hunt/Point/Retrieve, Pointers and Setters - although many of the breeds are capable of doing the same work as the other sub-groups. They make good companions, their temperament making them ideal all-round family dogs." https://www.thekennelclub.org.uk/activities/dog-showing/breed-standards/

[2] Hounds – "Breeds originally used for hunting either by scent or by sight. The scent hounds include the Beagle and Bloodhound and the sight hounds such breeds as the Whippet and Greyhound. Many of them enjoy a significant amount of exercise and can be described as dignified, aloof but trustworthy companion" https://www.thekennelclub.org.uk/activities/dog-showing/breed-standards/

[3] Terriers – "Dogs originally bred and used for hunting vermin. 'Terrier' comes from the Latin word Terra, meaning earth. This hardy collection of dogs was selectively bred to be extremely brave and tough, and to pursue fox, badger, rat and otter (to name but a few) above and below ground. Dogs of terrier type have been known here since ancient times, and as early as the Middle Ages, these game breeds were portrayed by writers and painters" https://www.thekennelclub.org.uk/activities/dog-showing/breed-standards/

[4] Toy – "The Toy breeds are small companion or lap dogs. Many of the Toy breeds were bred for this capacity although some have been placed into this category simply due to their size. They should have friendly personalities and love attention. They do not need a large amount of exercise and some can be finicky eaters." https://www.thekennelclub.org.uk/activities/dog-showing/breed-standards/

Walker, 2018). These locations feature an increased number of smaller calibre vessels and an increased predisposition to infarction (Holm et al., 2015). Injuries start with an erythematous and mild cutaneous swelling, situated around the tarsus, stifle, or inner thigh most commonly (Figure 4, 5 and 6) (Carpenter et. al.,1988). Less frequently, lesions can be found in the head with both cutaneous and oral lesions (Figure 7 and 8) (Skulberg et al., 2018). First opinion veterinarians often consider these lesions as a typical pyoderma, pododermatitis, bite/sting, or wound (Holm et al., 2015). These wounds were typically treated with systemic antimicrobial agents which did not appear to have any improvement on the skin lesion. The healing is indeed very slow, usually taking often up to two months (Carpenter et. al.,1988).

Figure 2 - Ulcerated lesion situated in the digital and metatarsal pads in a dog confirmed with CRGV. (Gently authorised by David Walker, Anderson Moores Veterinary Specialists).

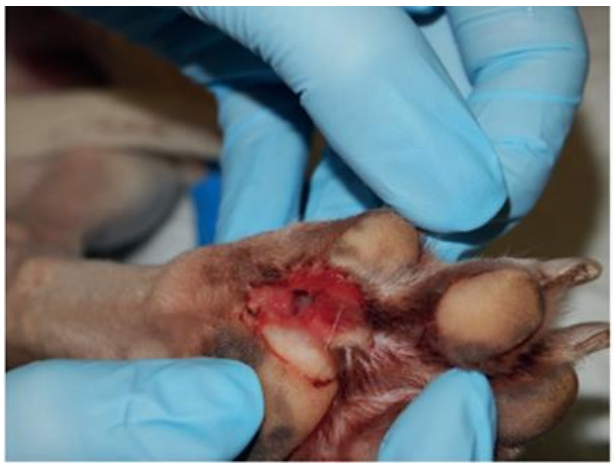


Figure 3 - Erosion to the carpal pad in a dog confirmed with CRGV (Gently authorised by David Walker, Anderson Moores Veterinary Specialists).

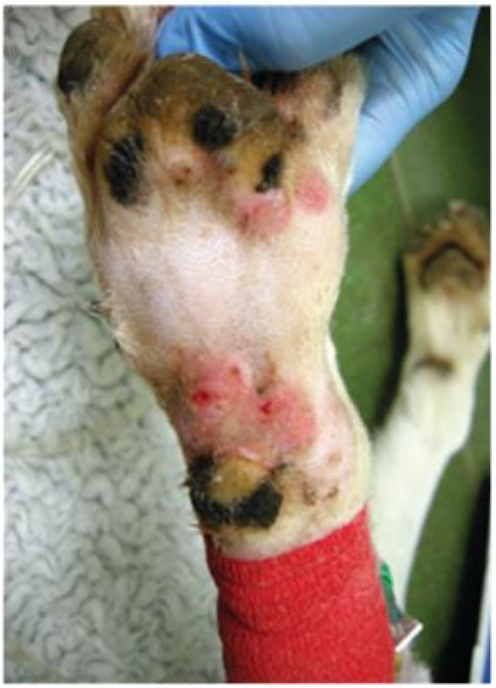


Figure 4 - Ulcerated lesion situated in the craniolateral thigh in a dog confirmed with CRGV. (Gently authorised by David Walker, Anderson Moores Veterinary Specialists).

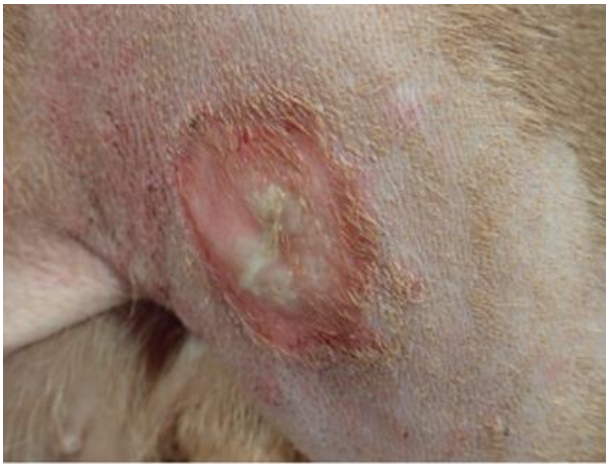
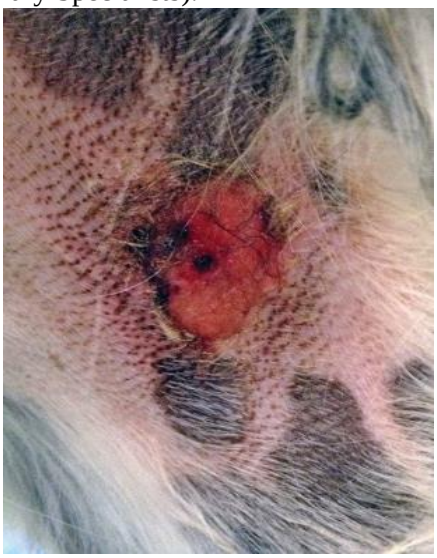


Figure 5 - Superficial ulcer located in the medial thigh in a dog confirmed with CRGV (Gently authorised by David Walker, Anderson Moores Veterinary Specialists).



According to the study conducted by Holm (2018) in 30 dogs that develop systemic involvement, including AKI, presented with anorexia (n=20), vomiting (n=20), lethargy (n=19), hypothermia (n=19), lameness (n=10), icterus (n=6), tachycardia, pyrexia (n=6), diarrhoea (n=4), petechiae (n=4), seizures (n=3), haematochezia (n=2), haematemesis (n=1), epistaxis (n=1), polyuria/polydipsia (n=1), ataxia (n=1) and behavioural changes (n=1) (Holm, 2015). Moreover, dogs can develop signs of volume overload (weight gain in comparison to the weight reported in the history when healthy, the presence of cutaneous oedema or cavitary effusion and/or peritoneal effusion) and some patients can be hypertensive (Skulberg et al., 2018).

Figure 6 - Ulcerated lesions found in the prepuce, caudal thighs and scrotum of a dog confirmed with CRGV (Gently authorised by David Walker, Anderson Moores Veterinary Specialists).

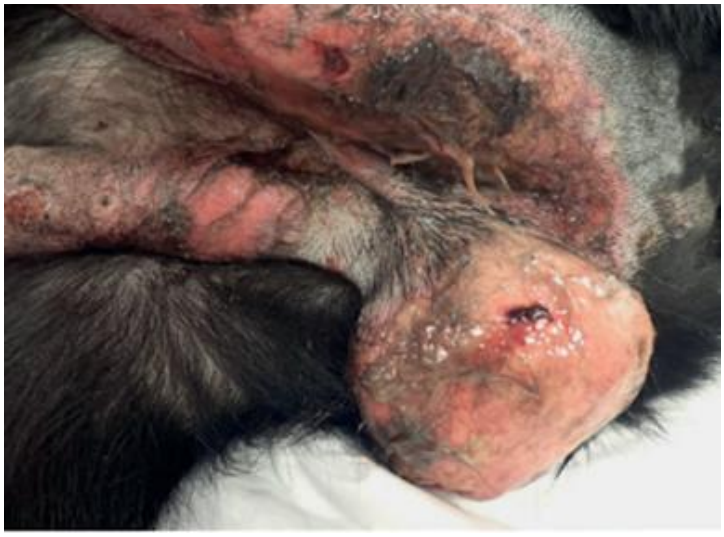


Figure 7 – Cheek superficial lesions in a dog confirmed with CRGV (Gently authorised by David Walker, Anderson Moores Veterinary Specialists)

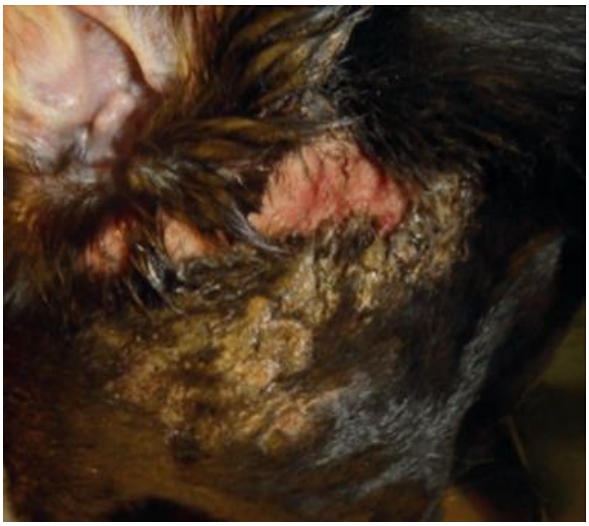
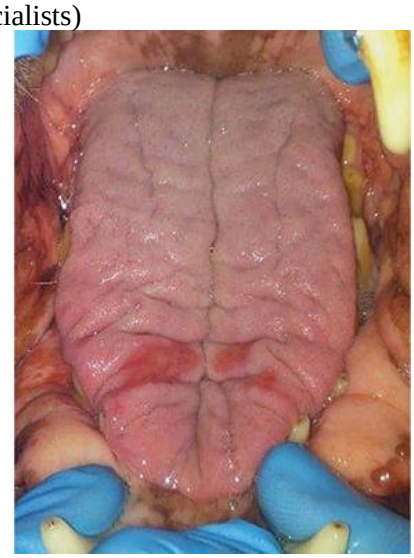


Figure 8 – Tongue lesions in a dog confirmed with CRGV (Gently authorised by David Walker, Anderson Moores Veterinary Specialists)



7. Clinicopathological findings

8.1 Haematological findings

A recent study, conducted by Holm & Walker (2018), described 52 percent of dogs with neutrophilia, 77.5 percent with thrombocytopenia and 22.2 percent with anaemia which is consistent to previous reports (Carpenter, et. al, 1988; Hertzke, et. al, 1995).

Dogs that have skin lesions, but do not consequently develop AKI are likely to have results within the normal range in haematology at the time of presentation, however mild neutrophilia can be found in some cases. A percentage of these patients will present with thrombocytopenia and/or anaemia. Contrary to these findings, dogs that develop AKI have at least one abnormality of packed cell volume/haematocrit, neutrophil or platelet count at the time of initial assessment (Holm & Walker, 2018). Typically, thrombocyte counts appear to decrease sharply immediately prior to the rise in serum urea nitrogen and creatinine (Hertzke et al., 1995; Holm & Walker, 2018).

Dogs affected with CRGV usually present with anaemia (Carpenter et. al.,1988) or became anaemic after presentation (Holm et al., 2015). The anaemia is pre- or non-regenerative. The possible aetiologies are gastrointestinal haemorrhage secondary to uraemia or microangiopathic red cell injury (Hertzke et al., 1995). Hypoalbuminaemia also occurs, and supports gastrointestinal haemorrhage, without excluding other possible causes (Holm et al., 2015).

Blood smears show evidence for burr cells, schistocytes or acanthocytes (Holm et al., 2015).

8.2 Biochemical findings

In general, dogs that do not develop clinically significant AKI have no biochemical abnormalities on initial presentation, however, a slight increase in the serum liver enzymes can be present: elevated alanine aminotransferase (ALT) and alkaline phosphatase (ALKP).

The dogs developing azotaemia at any point during their illness have raised serum urea concentration and serum creatinine concentrations (Holm et al., 2015). In the study conducted by Holm & Walker (2018), serum urea concentration and serum creatinine were elevated in 95.7 percent and 93.5 percent of the dogs, respectively, at the time of initial presentation. Hyperphosphatemia was observed in 78.3 percent of dogs, as well as hyperbilirubinemia, mildly elevated serum liver enzyme activities (ALT and ALKP), mildly elevated serum muscle enzyme activity, creatine kinase (CK) and aspartate aminotransferase (AST). Also, an abnormal specific canine pancreatic lipase may also be identified in 79 percent of the cases, but the significance of this finding is still unclear (Holm & Walker, 2018).

Dogs presenting with AKI should be evaluated according to the International Renal Interest Society (IRIS) AKI guidelines system. Most of these patients belong to Grade III (creatinine 221-439 µmol/l) and Grade IV (440-880 µmol/l) during the first 24 to 48 hours of hospitalization (Skulberg et al., 2018).

Standard coagulation profile including activated partial thromboplastin time (aPTT) and prothrombin time (PT) are within normal reference intervals (Skulberg et al., 2018).

8.3 Urinalysis

The results of the urinalysis of dogs with CRGV with AKI are consistent with results found in patients with AKI of any cause (Piech & Wycislo, 2019). The abnormalities commonly detected at initial presentation include proteinuria, median urine protein: creatinine ratio (UPC) 3.42 (range 1.81 to 7.64; reference range <0.5), haematuria/haemoglobinuria, glucosuria, and urinary casts, mostly granular or hyaline. Urine specific gravity can vary but its median is 1.015 which according to IRIS corresponds to a minimally concentrated urine specific gravity. Patients that do not develop clinically AKI have generally unremarkable urinalysis, except for some cases which can present mild proteinuria (Holm & Walker, 2018).

8.4 Imaging

When haematology, biochemistry and/or urinalysis detect any abnormalities, it is always useful to pursue further diagnostics. In azotaemic cases, ultrasonography (US) is a useful tool for

excluding other causes of AKI. In general, ultra-sonographic findings in animals affected by CRGV are unremarkable. Some animals present hyperechoic renal cortices. Free abdominal fluid due to volume overload or haemorrhage can be found (Holm & Walker, 2018). Abdominal US findings more commonly reported are peritoneal effusion as well as, enlarged and oedematous pancreas with mixed echogenicity. Thoracic radiographs report no specific abnormalities on intrathoracic structures (Skulberg et al., 2018).

8. Definitive diagnostic

The definitive and accurate diagnosis of CRGV is only via renal histopathology, and there is no single test available which can predict which dogs will develop clinically significant AKI. Veterinarians, however, must be aware of this illness if multiple skin lesions are present, with extensive oedema and/or bruising, systemic signs like lethargy, pyrexia, anorexia, and presence of laboratory abnormalities such as anaemia, neutrophilia, thrombocytopenia, hyperbilirubinemia, and proteinuria, should complement to build a strong suspicion regarding the development of AKI. Nevertheless, some dogs have innocuous lesions and no other laboratory abnormalities and still develop AKI. This inconsistency leads to a challenging antemortem diagnosis. If there is a high suspicion of CRGV, based on time of the year of the lesion display, then further laboratory tests such as haematology, biochemistry, and a urinalysis may provide more useful information that supports the diagnostic, and a baseline for ongoing monitoring. If all these results are unremarkable, then just monitoring is the appropriate procedure in most cases (Holm & Walker, 2018). The dogs suspected with CRGV were all diagnosed at post-mortem analysis, as there are concerns about the invasiveness of renal biopsy in patients with AKI (Ross, 2011). One of the most common complication of renal biopsy is haemorrhage (DiBartola, 2010). In addition, patients with severe azotaemia, which occurs in CRGV, are more likely to have major complications such as severe haemorrhage and hydronephrosis. Abnormal bleeding in patients with uraemia is described by increased bleeding time and platelet-function abnormalities (Vaden et al., 2010).

9. Histopathology

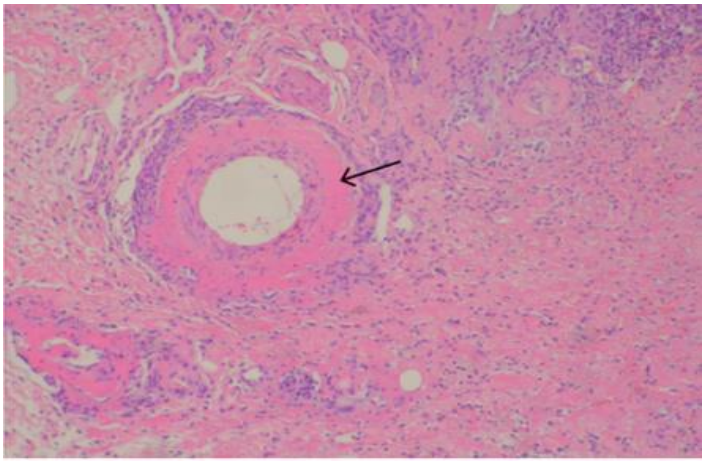
CRGV is a disease reported to cause ulceration of the distal extremities in dogs. It is variably associated with clinically acute kidney injury, so the most remarkable and characteristic lesions are allocated to the skin and kidneys (Holm et al., 2015). Previous studies, however, demonstrate that macroscopic lesions can occur in other organs as well, and can include: ascites and/or pleural effusion, haemorrhages on serosa surfaces, mural oedema of the stomach, small

intestine, and/or colon, ulcerative glossitis, red-black flecked gastric and small intestinal contents and tarry colon contents (Hertzke, et. al, 1995). The microscopic changes of these structures reported in this study consisted of rare thrombi in submucosal arterioles of the stomach, small intestine, and colon; small intestine crypts can be occasionally dilated and filled with necrotic debris and neutrophils; heart had widely scattered fibrinoid vascular necrosis, with rare foci of myocardial necrosis; hemosiderin inclusions in macrophages were found commonly within lymph nodes, liver, and spleen. This diffuse finding can be a nonspecific indicator of increased erythrocyte destruction that may result from the elimination of damaged erythrocytes, as occurs in microangiopathic processes; central nervous system lesions were limited to the mild expansion of peri-vascular spaces (Hertzke et al., 1995). Yet, in the study conducted by Holm et al. (2015), other tissues were evaluated and analysed, and changes appeared unremarkable, with mild and non-specific changes.

Histopathology of the skin samples is not a reliable diagnostic tool for CRGV. Most commonly the findings are non-specific, even though skin biopsies may be diagnostically useful as a means to reject other differential diagnostics, such as immune-mediated and neoplastic diseases. (L. Holm & Walker, 2018). Multifocal subcutaneous haemorrhages, oedema, and multifocal fibrinoid necrosis of small to medium-sized arterioles, with abrupt, full-thickness necrosis of the epidermis were found, and this suggests that cutaneous vasculitis with ischemic necrosis is a possible pathogenic mechanism (Hertzke et al., 1995).

The subcutaneous and deep dermal arterial lesions varied from mild to severe and ranged from increased eosinophilia of the tunica media to pyknosis, karyorrhexis and occasionally fibrinoid necrosis (Figure 9) (Carpenter, et. al, 1988), with, rare fibrinocellular thrombi. The subjacent dermis was often undergoing coagulative necrosis (Holm et al., 2015).

Figure 9 - Histopathology of a skin biopsy from a patient with CRGV, showing a dermal artery with fibrinoid necrosis (arrow). Haematoxylin and eosin, x100. (Gently authorised by David Walker, Anderson Moores Veterinary Specialists).



At the level of the adnexa, the affected hair follicles are necrotic in the entire pilosebaceous units, leading to reduced or absent sebaceous glands, reduce cellularity and separate by an increase fibrous tissue and an attenuate follicular epithelium (Figure 10 and 11). The affected follicles were often bordered by variable numbers of neutrophils, foamy macrophages, and karyorrhectic debris; this often obscured the follicular epithelium interface and sebaceous gland units. (Hertzke et al., 1995; Holm et al., 2015).

Figure 10- Necrotic hair follicle along with neutrophilic infiltrates. (Gently authorised by David Walker, Anderson Moores Veterinary Specialists)

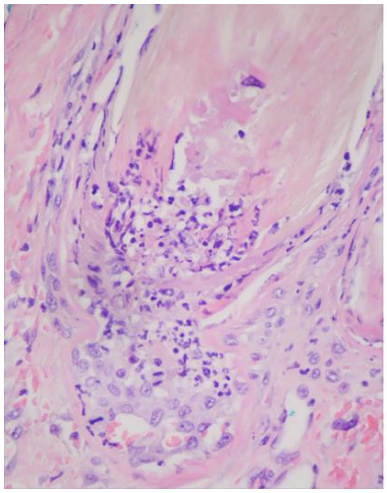
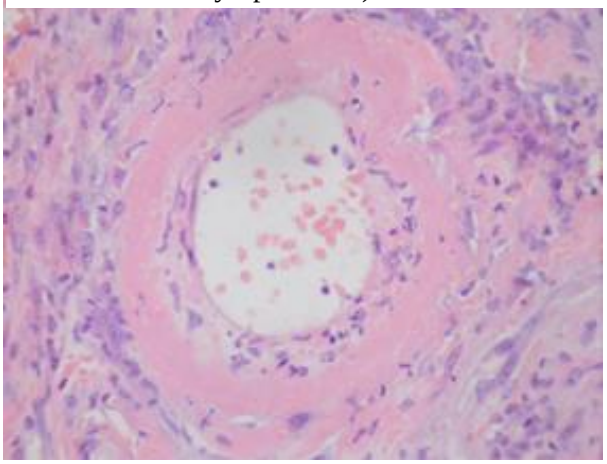


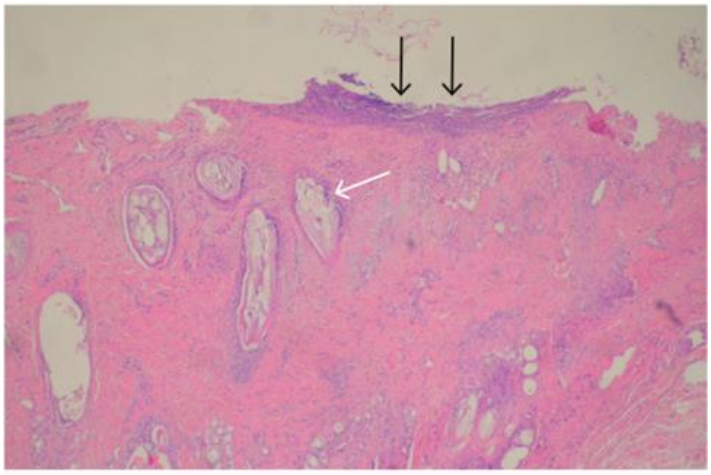
Figure 11 - Photomicrograph of small dermal artery with fibrinoid necrosis of the vessel wall (Gently authorised by David Walker, Anderson Moores Veterinary Specialists)



In samples from the oral cavity lesions, similar ulceration of the mucosa was observed with associated necrosis, inflammation and fibrovascular change of the submucosa (Holm et al., 2015).

In conclusion, skin biopsies are not a reliable method to confirm the definitive diagnostic of CRGV but are a very helpful tool towards the final diagnosis and choice of treatment. The biopsies reveal necroulcerative dermatitis with vasculitis and thrombosis. The principal findings include extensive dermal ulceration with necrotizing folliculitis and perifolliculitis and adnexal necrosis with occasional vasculitis. The lesions were mostly centred on the hair follicles and adnexa (Figure 12) (Skulberg et al., 2018).

Figure 12 - Histopathological appearance of a skin biopsy from a patient with CRGV. Haematoxylin and eosin, x40. (Gently authorised by David Walker, Anderson Moores Veterinary Specialists).



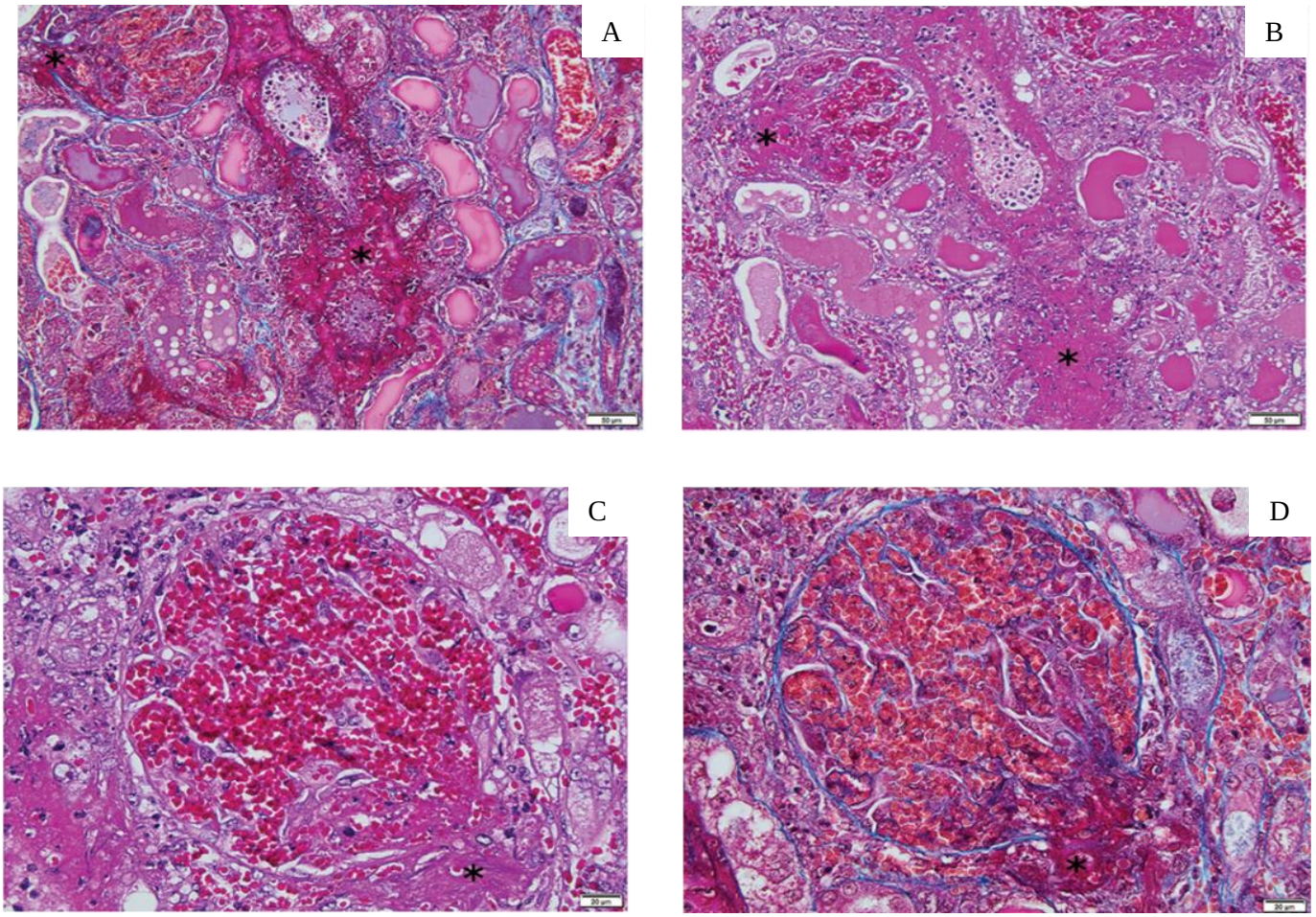
Caption - This is haired skin demonstrating extensive ulceration (black arrows) and necrosis of the adnexa, with secondary pyogranulomatous inflammation near disrupted hair follicles (white arrow).

Macroscopically, kidneys are uniformly swollen, unremarkable or slightly pale, congested, with numerous, irregularly distributed, barely visible, cortical and capsular petechiae (Carpenter, et. al, 1988; Hertzke et al., 1995).

The most consistent and severe microscopic lesions were found in the kidneys (Hertzke et al., 1995), with the most striking changes involving the glomeruli (Holm et al., 2015).

Carpenter et al. (1988) found in all kidneys of the greyhound interstitial congestion, oedema, and small haemorrhages originating at or near glomerular vascular poles. This specific area and adjacent to interlobular and intralobular veins a mild, multifocal, lymphocytic, and plasmocytic interstitial infiltrate were present. Most of the glomeruli are affected and for individual glomeruli, these changes range from mild and segmental to global and severe (Holm et al., 2015). These changes consist of hyaline thrombi within capillaries and afferent arterioles, segmental to global congestion and ischemic necrosis (Hertzke et. al, 1995). Fibrinoid necrosis occurs frequently and is defined by a distortion of vessel walls with eosinophilic, hyalinised, smudgy material, intermingled with low numbers of degenerate and viable neutrophils, fragmented red blood cells and mild amounts of karyorrhectic debris (Holm et al., 2015). Glomerular tufts are enlarged and congested, partially occluded by haemorrhage, obliterating the urinary space, and are mildly hypercellular. Glomerular capillary walls are thickened by finely fibrillar material. A minority of glomeruli are small, shrunk, and hypocellular. The interstitium has scatter periglomerular haemorrhages and mild oedema. Medullary congestion is also present. (Hertzke et al., 1995; Holm et al., 2015). Tubular changes are multifocal and can alter in extent (Hertzke et al., 1995) which include, hypoxic nephrosis, striking hyaline droplet change, and a variety of casts (hyaline, granular, bile-stained, and red blood cell) (Figure 13) (Carpenter et. al, 1988).

Figure 13 (A, B, C and D)- Photomicrographs of a glomerulus and an intralobular artery from a dog with CRGV (Gently authorised by David Walker, Anderson Moores Veterinary Specialists)



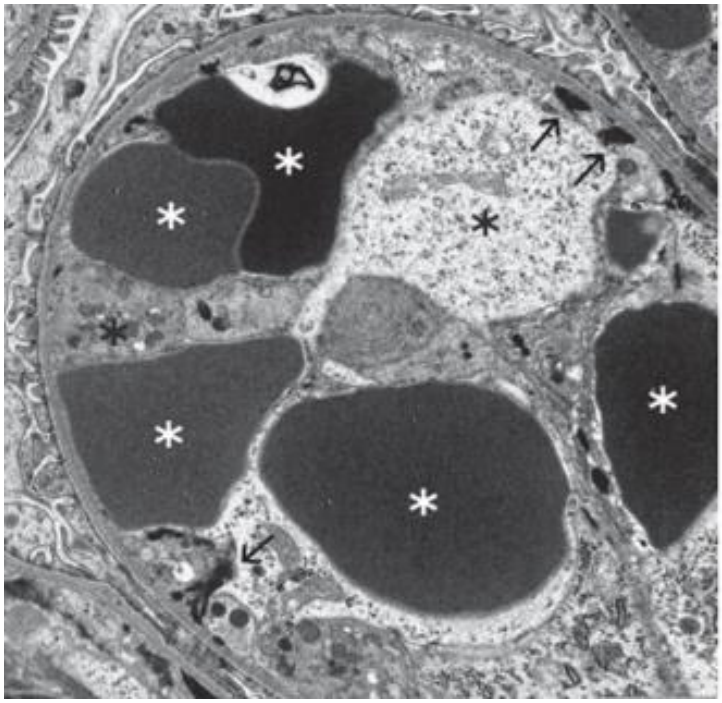
Caption: Fibrinoid vascular necrosis (asterisks) of intralobular arteries and arterioles. Glomerular capillaries are extremely distended and contain red blood cells, most of them fragmented. Distension of glomerular capillaries is due to dissolution of the mesangial matrix (mesangiolysis). Tubules are suffering degeneration and necrosis. Most of these structures contain protein casts although some contain red blood cell casts.

10. Electron microscopy

Electron microscopy of the kidneys, more precisely of the glomeruli, reveal the expansion of glomerular capillary loops by erythrocytes, occasional schistocytes, and rare polymorphonuclear cells. Endothelial cells, when identifiable, are severely swollen. Occasionally, mesangiolysis (dissolution of mesangium) is notable. Immune complexes are not detectable. (Holm et al., 2015). Hertzke (1995) was able to demonstrate and characterize the sequential changes in glomeruli of Greyhounds with CRGV and could be divided into early, middle, and late changes, and interrelated with clinical findings. The *early stages* can be verified during the pre-azotaemia stage, immediately preceding the onset of thrombocytopenia - glomerular endothelial cells often are focally deprived of the basement membrane.

Approximately a small number of these cells are necrotic with condensed nuclear chromatin and membranous whorls. More endothelial changes consist of fragmentation, cytoplasmic swelling and vacuolization, and loss of fenestrations. Platelets adhere and aggregate in the areas where the basement membrane was exposed. These aggregates of platelets with a diverse amount of fibrillar material consistent with fibrin, cellular fragments, erythrocytes, and rare neutrophils can occlude some capillaries. Occasionally fusion of the visceral epithelial foot processes was seen. The basement membrane and mesangial cells are unchanged; *Middle changes* were taken from most Greyhounds with AKI and submitted to euthanasia. These changes are defined as enlarged endothelial cells, with increase rough endoplasmic reticulum, occasional cytoplasmic lipid, few fenestrations, and villous cytoplasmic processes. Endothelial cell nuclei appear to come close to when compared with healthy Greyhound glomeruli. Glomerular capillary lumina are recurrently narrowed, and the capillary walls are thickened because of the expansion of subendothelial space by amorphous to finely granular, variably electron-dense material. Cytoplasmic processes, some with dense fibrillar areas subjacent to the plasma membrane typical of mesangial cells, can be found interposed between the basement membrane and endothelial cells. In some glomeruli, the expanded subendothelial spaces contain cellular fragments, erythrocytes, fibrin, and cells of undetermined origin (Figure 14). The lamina rara externa and lamina densa remained relatively unchanged. The fusion of the visceral epithelial foot processes is seen variably; during recovery, clinical evidence of renal failure can be absent (*later changes*) but ultrastructural changes are similar but less severe. Usually, we can find hypertrophy of the endothelial cell, villous cytoplasmic projections from endothelial cells into the capillary lumen, and interposition of mesangial cell cytoplasmic processes into the subendothelial spaces. Glomerular capillary lumina is narrowed and contains few circulating red blood cells. Irregular wrinkling of the basement membrane occurs in some glomeruli. In dogs that do not present with clinical apparently relevant renal disease, glomerular changes are multifocal, mild, and predominately endothelial. These include endothelial hypertrophy and proliferation and villous transformation of endothelial cytoplasm. Cells are occasionally in mitosis. Rarely, mild expansion of the subendothelial space is present. These changes reveal that subclinical renal involvement occurs; however they were less severe and not as widespread as those in Greyhounds with renal insufficiency (Hertzke et al., 1995).

Figure 14 - Electron micrograph of a glomerular capillary loop from a dog with cutaneous and renal glomerular vasculopathy (Gently authorised by David Walker, Anderson Moores Veterinary Specialists).



Caption: Multiple deformed red blood cells (white asterisks); swollen endothelial cells (black asterisks); small aggregates of fibrin tactoids at the periphery of the capillary loop (black arrows).

11. Medical management

12.1 Management of cases

12.1.1 Without apparent clinically relevant AKI

Adequate management and monitoring of skin lesions and renal function are imperative if initial investigations confirm that there is no evidence of AKI. In the euvolaemic patient, there is no evidence-based that fluid therapy in patients at risk of developing AKI, changes the outcome. It is also important to acknowledge that, as fluid accumulation dilutes serum creatinine concentration, administration of intravenous fluids may postpone an early detection stage of AKI if depending exclusively on this parameter. A correction factor has been formulated to allow for the effects of fluid accumulation on serum creatinine concentration (Macedo et al., 2010). Nevertheless, fluid therapy may be necessary to avoid the development of dehydration or hypovolaemia, if vomiting or diarrhoea are present. Special attention should be taken to avoid the use of certain drugs that are known to increase the risk of AKI such as non-steroidal anti-inflammatory drugs (NSAIDs). Likewise, there is no evidence that supports the administration of drugs for thrombi prophylaxis, or steroids at immunosuppressive or anti-inflammatory doses (Holm & Walker, 2018).

12.1.2 With apparent clinically relevant AKI

When AKI is confirmed through laboratory analyses, the medical approach consists on the exclusion of other known causes, before assuming CRGV is present. CKD and post-renal causes should be excluded firstly. After their exclusion, other potential causes of AKI must be considered, for example, potential nephrotoxins, full urinalysis including culture should be performed, leptospirosis testing (PCR on blood/urine before antibiotic therapy and microscopic agglutination test), abdominal imaging to assess renal architecture, analysis of serum electrolyte concentrations, and a basal cortisol blood test/ACTH stimulation test (to exclude hypoadrenocorticism) should all be considered (Holm & Walker, 2018).

CRGV is a disease where high mortality is shown when AKI develops. Therefore, it is crucial that intensive care is provided to these dogs. Intravenous fluids, electrolytes, vitamins and parenteral alimentation for two to five days allowed 25 dogs in Carpenter's study to survive and overcome the illness (Carpenter et al., 1988).

These patients have haematological irregularities and their consequences must be addressed, along with nausea, pain and nutritional necessities (Holm & Walker, 2018).

12.1.2.1 Fluid therapy

Because of the importance of the role of the kidneys in maintaining homeostasis, kidney failure may lead to abnormalities of fluid, electrolyte, acid-base balance and appropriate changes in the treatment plan may be needed in response to the rapidly changing clinical situation of the kidney disease patient. Reversing these imbalances is the aim of treatment.

In fluid therapy, body losses must be considered. Normal fluid losses consist of insensible and sensible losses. Insensible losses are water lost via respiration, normal stool, or sweating. The main sensible fluid loss in the healthy patient is urine output. Further sensible losses include the volume lost from vomiting, diarrhoea, and others (Langston, 2017a).

If hypovolaemia is present it must be amended promptly, via administration of a 20 ml/Kg bolus of crystalloids, given over ten minutes, which can be repeated if necessary. After the bolus, a 5 percent dehydration deficit should be considered in all dogs with AKI and must be improved over four to six hours. Maintenance and ongoing loss requirements must also be adjusted (L. Holm & Walker, 2018). A balanced polyionic solution (e.g. lactated Ringer solution [LRS], Plasmalyte-148, Normosol-R) is a suitable choice for the initial volume resuscitation fluid and replacement of the dehydration deficits. Physiologic NaCl 0.9% does not contain potassium and is an appropriate initial choice for the hyperkaliaemic patient. After rehydration, maintenance fluids with a lower sodium concentration are considered a better option (e.g., 0.45% NaCl with 2.5 percent dextrose, one-half strength LRS with 2.5 percent

dextrose) (Langston, 2017a). After the initial fluid resuscitation, the volume of fluid to administer is calculated by adding average maintenance fluids (66 mL/Kg per day) plus replacement of dehydration (in patients with AKI, a 5 per cent dehydration is always considered) plus ongoing losses (e.g., the estimated volume of polyuria, vomiting). The fluid rate should be adjusted based on on-going estimates of patient volume status (Langston, 2017a). When the urine output (UOP) is within the normal range, fluid therapy should be continued matching 'ins and outs' and serum electrolyte concentrations should be closely monitored. Changes of sodium concentration need to be avoided, due to the potential neurological consequences, as well as potassium (development of hypo- and hyperkalaemia). This management should only be discontinued when the serum creatinine concentration has improved to a plateau (Holm & Walker, 2018).

12.1.2.2 Urine Output

With renal disease, urine volume is can be abnormally high (polyuria) or low (oligoanuria). The UOP in a healthy dog is 1 to 2 mL/Kg/hr. A decrease in this volume may represent an appropriate renal response to hypovolemia or a pathologic change in renal function, which can lead to oliguria (urine production <0.5 mL/Kg/hr) or anuria (no urine production over six hours) (Langston, 2017b). As soon fluid therapy starts urine production should increase to 2 to 5mL/Kg/h (Ross, 2011).

Placement of an indwelling urinary catheter with a closed collection system is the most effective method of assessing the quantity of urine produced. If this procedure is not possible, urine output can be evaluated through catching and measuring urine in containers when the patient is taken outside or by placing incontinence pads in the kennel which can be weighed. Both methods become very untrustworthy if oligoanuria develops. Successive weight measurements can also be taken to guarantee that the patient does not become either overhydrated (volume overload) or dehydrated (Ross, 2011). Urinary bladder size can be measured ultrasonographically if the urine output seems subjectively reduced. Nevertheless, this will not enable an accurate definition of whether the patient is oliguric or anuric. Notwithstanding, a recent study proved that using a simple, point-of-care ultrasonographic cysto-colic view for estimation of urinary bladder volumes using the current simple equation, carries the potential to be clinically useful for non-invasive estimation of urinary bladder volume and thus calculation of UOP in both dogs and cats (Lisciandro & Fosgate, 2017).

If polyuria progresses and fluid requirements are not met, there is a risk of hypoperfusion and further renal injury (Holm & Walker, 2018). If the UOP does not improve solely with fluid therapy and the patient is well-hydrated, medical management for oliguria should be initiated.

The diuretics used can include furosemide, conventionally initially administered as a bolus (2 mg/Kg). If this does not show an improvement of urine production within an hour, single boluses (4 mg/Kg, then 6 mg/Kg) should be administered. If it is effective, treatment can be continued with a constant rate infusion (0.3 to 1 mg/Kg/hour) for 24 to 48 hours; mannitol (osmotic diuretic), if furosemide is ineffective. It is initially administered as a 0.25 to 0.5 g/Kg bolus of 10 or 20 per cent solution. If this is effective, a constant rate infusion (1 to 2 mg/Kg/min) can be continued for 12 to 36 hours. Mannitol should be used with special caution in oligoanuric patients as it can worsen circulatory overload; if medical management fails to improve the patient condition and treatment is to be continued, dialysis will be required to correct water, electrolyte, and acid-base abnormalities and to remove uraemic toxins. Referral for continuous renal replacement therapy can be considered and advised to the pet owner (Holm & Walker, 2018).

12.1.2.3 Medication

Hypertension in AKI patients should always be considered (IRIS, 2016). If the systolic blood pressure persistently goes above 160-179 mmHg there is a bigger risk of retinopathy and choroidopathy, heart and blood vessels and CNS damage, and progression of the renal disease. Renin-angiotensin-aldosterone system inhibitors (angiotensin-converting-enzyme inhibitor (ACEi), angiotensin II receptor blockers (ARBs), and aldosterone antagonists) and calcium channel blockers (CCB) are the most extensively recommended antihypertensive agents to use in dogs. Because of their antiproteinuric effect and the high prevalence of CKD in hypertensive dogs, renin-angiotensin-aldosterone system (RAAS) inhibitors are often chosen as first-line antihypertensive agents. Within this group an ACEi (e.g. 0.5-2.0 mg enalapril or benazepril/ Kg PO q12h). As an alternative pathway for RAAS inhibition, an ARB (e.g., 1.0 mg telmisartan/Kg PO q24h) can be used. The use of CCB as monotherapy in dogs should be avoided because CCB dilates the renal afferent arteriole potentially exposing the glomerulus to damaging increases in glomerular capillary hydrostatic pressure (Acierno et al., 2018).

Antimicrobials should be given for the prevention of bacterial infections and in the event the pathogenesis could be related to an infectious cause, as it is still unknown (Skulberg et al., 2018). Therefore, the benefits of using these drugs are unclear. These should only be used if there is evidence of skin infection, urinary tract infection, or those considered at high risk for developing these complications (such as patients with neutropenia) (Holm & Walker, 2018). In human medicine, one study proved that there is a significant positive correlation between antibiotic administration and the risk of developing HUS (Freedman et al., 2016).

Nausea can be severe in patients with CRGV (Holm & Walker, 2018) thus anti-emetic therapy

should be provided such as maropitant (2 mg/Kg PO q 24 h or 1 mg/Kg SC q 24 h for 5 days), metoclopramide (0.1-0.5 mg/Kg PO, SC, IM q 6-8 h, 0.01-0.02 mg/Kg/h CRI), ondansetron (0.1 mg/Kg PO q 12-24 h) or dolasetron (0.5 mg/Kg PO SC, IV q 24 h). These two last drugs can be used in patients whose symptoms are not controlled with maropitant and metoclopramide (Langston, 2017).

Increased gastric acid production secondary to renal dysfunction can lead to uraemic gastritis. Drugs that reduce gastric acid production may, therefore, be beneficial as part of the management for patients with AKI: Omeprazole, a proton pump antagonist (dose 0.5 to 1.5 mg/Kg PO or IV twice a day) and Famotidine, H2-receptor antagonist (dose 0.5 to 1.0 mg/Kg administered PO once to twice a day) (Holm & Walker, 2018)

Pentoxifylline increases erythrocyte flexibility, fibrinolysis, and diminishes blood viscosity, therefore can be administered as a potential adjunctive treatment for the presumed presence of vasculitis, one feature of CRGV (Skulberg et al., 2018; Holm & Walker, 2018)

Analgesia is required for pain management as CRGV is an extremely painful condition (Skulberg et al., 2018; Jepson et al., 2019). The safest drugs for the pre-anaesthetic medication of patients with AKI are opioids and benzodiazepines, due to minimal effect on the cardiovascular system, ability to decrease the dose of other anaesthetic agents and can be antagonized. The pain score must be assessed, and an analgesic drug chosen accordingly. If the pain is severe then full μ opioid receptor agonists such as methadone or morphine (0.2-0.3 mg/Kg i.m. or i.v.), must be administrated; if the pain is mild, butorphanol (0.2-0.3 mg/Kg i.m. or i.v.) or buprenorphine (0.02 mg/Kg i.m. or i.v.) can be considered. It is vital to control pain appropriately, as untreated pain will stimulate the sympathetic nervous system stress response, which may lead to further renal hypotension (Garcia, 2016).

The ideal diet for AKI is not known. In the absence of this data, enteral diets for critically ill animals or people have been used (Langston, 2017a).

12.1.3 Wound management

Wound management should be initiated once the animal is clinically stable. If the patient has complications such as hyperkalaemia and uraemic crisis, these must be stabilised over at least 24 hours before anaesthesia is an option (Jepson et al., 2019), but analgesia should be provided before handling the patient (Devriendt & de Rooster, 2017).

Before any topical or systemic antimicrobial therapy is initiated, samples for cytology and bacteriology must be collected. Cytology can be used to distinguish between contamination by commensals and real pathogens, bacteriology will identify the potential pathogen and sensitivity testing will designate the most appropriate antimicrobial for each case (Devriendt &

de Rooster, 2017). If the culture results are positive for a microorganism, systemic antimicrobial therapy is only required if there are clinical signs that indicate infection (Bowler, Duerden, & Armstrong, 2001).

Lesions that occur in CRGV seldom require debridement (Dissemond et al., 2013), so the next step will be lavage with a high volume of phosphate-buffered saline or lactated Ringer's solution delivered via a large syringe and 18-gauge catheter (Liptak, 1997). After this procedure, a sterile dressing should be applied, to work as a physical barrier to prevent contamination and infection and to accelerate wound healing. This will maintain a moist wound environment (Tobias, 2012). This environment is considered beneficial because it is thought to limit the chance for infection, as more neutrophils are present in moist wounds because these are not trapped in scabs, and epithelialization ensues more promptly because epithelial cells do not need to migrate underneath a scab. Furthermore, wound fluid is thought to enable autolytic debridement of necrotic tissue due to endogenous enzymes. This fluid likewise contains cytokines and growth factors that stimulate fibroplasia and epithelialization, reliant on the phase of wound healing. Wound fluid may also contain antibiotics if the animal is receiving systemic antibiotics (Balsa & Culp, 2015).

12.1.4 Monitoring

Monitoring dogs with suspicion of CRGV consists in daily/alternate day assessment of routine haematology, biochemistry and urinalysis, and assessing urine output (Holm & Walker, 2018), one of the most important and probably the most neglected aspects of monitoring animals with AKI (Ross, 2011).

AKI develops up to at least four to nine days after the appearance of skin lesions, so monitoring should cover this high-risk period. The clinical decision of monitoring needs to be supported with further data, such as clinical signs, physical examination, patient temperament, owner and clinician concern and financial considerations (Holm et al., 2015).

12.1.5 Therapeutic Plasma Exchange

The success rate with plasma treatment in adults with TTP/HUS is 90 percent. A disease which was unvaryingly fatal before, PEX has had a major effect on the treatment of most primary and secondary forms of TTP⁄HUS (Clark, 2012).

In patients with D+HUS, PEX has not been sustained by randomized trials and did not show any improvement. Although all human patients suspected to have aHUS or TTP should first be treated by plasmapheresis and/or frozen-plasma infusions. This therapy should be initiated as soon as possible, after presentation, which implies that the diagnosis must be fast as well, to

avoid mortality. Adults who present with inexplicable thrombocytopenia and a microangiopathic haemolytic anaemia with a normal International Normalized Ratio (INR) and PTT should immediately start on plasma infusion with urgent transfer (minutes to hours) to a plasma exchange unit for therapy (Clark, 2012).

Plasma exchange should be performed together with fresh-frozen plasma infusion. Indeed, plasma exchange removes factors involved in the pathogenesis such as CFH, CFI, CFB and C3 mutated in addition to anti-factor H antibodies, an infusion of frozen fresh plasma regularizes levels of CFH, CFI, CFB, and C3. Furthermore, plasma exchange associated with plasma infusion avoids the volume overload. Plasma therapy should be performed as frequently as possible, with PE at 1.5 plasma volumes per session. Plasma therapy should be performed until a normal platelet count, haemoglobin and LDH have been achieved (Salvadori & Bertoni, 2013).

Based on the potential resemblance in the pathophysiology amongst canine CRGV and conditions in people such as TTP or aHUS, PEX was hypothesized as a new therapeutic option for affected dogs (Walker, Holm, Hawkins, & Cianciolo, 2014).

In 2018, Skulberg et al., revised and compiled six cases of dogs diagnosed with CRGV (n=2 antemortem, n=4 post-mortem) that underwent to at least one cycle of plasma exchange (PEX) (Skulberg et al., 2018). All patients presented with cutaneous lesions and were azotaemic with oliguria or anuria at the time of treatment. Difficulties seen during PEX therapy included hypothermia, tachycardia, hypotension, and hypocalcaemia (observed in all dogs in this study). Two dogs survived to discharge, and the remaining four dogs were euthanized. The excellent outcome in two dogs treated with PEX despite the described high mortality rate once acute kidney injury with oliguria/anuria occurs, unfortunately, does not endorse the success of this therapy. Still, survival in two dogs that were primarily oligoanuric reinforces that special attention and evaluation of PEX is necessary for this specific disease and more studies are required, regarding the aetiology and treatment of CRGV (Skulberg et al., 2018).

12.Prognosis

If the patient does not develop AKI, in CRGV, the prognosis is considered excellent and full recovery is expected, however, skin lesions might take many weeks to months to heal completely, often dogs will be left with scars. The prognosis is fair if patients have AKI grade I to II (IRIS), and either not oligoanuric, or oliguric but responding well to medical management. For cases developing more clinically significant AKI (IRIS grades II to V; with oligoanuria responding poorly to medical management), the prognosis is severe with mortality around 90 percent (unpublished data, Anderson and Moores Veterinary Specialists). It is clear

to identify that 79 percent of the cases that were euthanized, for many different reasons such as, poor prognosis, financial constraints or concomitant diseases, which means these cases were not treated with continuous renal replacement therapy or TPE, enhancing the chances of full recovery. Even though in this study, nine suspected cases with IRIS AKI grades II to IV survived with intensive treatment (Holm & Walker, 2018).

II. Experimental work

1. Introduction and objectives

CRGV is a disease known since 1988 in the United States of America, with increasing occurrence in the UK since 2012 (Holm et al., 2015). This disease usually manifests itself with acute erythema and oedema progressing rapidly to cutaneous ulcers of the extremities, thrombocytopenia and clinically relevant acute renal failure (AKI). When acute renal failure develops it is usually fatal (Carpenter et al., 1988).Veterinary surgeons and owners across the UK are becoming more aware of this condition, and more cases are recognised every year. What causes this illness is still unknown (Jepson et al., 2019), however signalment risk factors have been previously studied (Stevens, 2018). CRGV is a TMA of unknown aetiology which, when azotaemia develops, appears to carry a severe prognosis when AKI is present. This study aims to review cases of dogs throughout the UK, suspected with cutaneous and renal glomerular vasculopathy with or without AKI describing their history and clinical signs, clinicopathological findings, diagnostics, treatment plan, and outcome and to correlate whether AKI is related with the skin lesions size and macroscopic characteristics and to a possible worse prognosis.

2. Materials and Methods

2.1 Case selection criteria

A search of computerised record systems, using keywords such as Alabama Rot and CRGV, was carried out at 26 first opinion emergency providers practices all over Great Britain (United Kingdom, Scotland, and Wales). Inclusion criteria were dogs diagnosed with skin lesions attributed to CRGV suspected cases, with or without clinically confirmed AKI of unknown aetiology, established through historical and laboratory evidence with or without clinical oligoanuria/anuria, anaemia and thrombocytopenia with subsequent review of clinical case files in order to identify cases compatible with CRGV.

2.2 Medical records review

Data were retrospectively collected from 26 first opinion emergency practices all over the UK, named VetsNow. Medical records were assessed by the veterinarians of these practices involved in the cases and sporadically through interview and personal communication. Clinical variables included: signalment (age, sex, reproductive status, weight, breed, location of the

clinic), relevant clinical history (from primary general practice and current emergency service), clinical signs, physical examination, clinicopathological findings (hematological and biochemical results, coagulation profiles, urinalysis), diagnostic imaging (US), medical management, and outcome. The postcode of each clinic, where dogs were seen by their emergency practices were listed on a map using Google maps (Figure 15).

Dogs were divided into two groups according to the AKI diagnosis: CRGV-SL (group of dogs presenting only with skin lesions and without AKI) and CRGV+AKI (dogs with skin lesions and clinically diagnosed AKI). AKI diagnosis was stablished when urea and creatinine were elevated and oligoanuria was present.

2.3 Laboratory diagnostics

The Minimum Data Base is a standard package of tests. These tests provide a baseline against which future monitoring as appropriate from the following: packed cell volume (PCV) using micro-haematocrits tubes following centrifugation and measurement; total solids (TS) is measured by breaking the haematocrit tubes (once the PCV is determined) after centrifugation, and placing the plasma directly onto the refractometer; blood lactate using the EDGE Lactate Monitor; blood glucose level, using a glucometer; and blood urea nitrogen (BUN) using Diasys Blood Urea Test Strips. Combur-Test® strips were used for the urinalysis. RapidBac™Vet which is a lateral flow immunoassay was used for the detection of Gram-positive and Gram-negative bacteria in the urine. EPOC® BGEM Test Card was used for the blood gases and electrolytes. It analyses pH, partial pressure of carbon dioxide (pCO_2), sodium (Na^+), potassium (K^+), ionized calcium (Ca^{++}), chloride (Cl^-), glucose (Glu), lactate (Lac), BUN/Urea and creatinine. For the assessment of kidney function of the animals in the group CRGV-SL blood urea nitrogen (BUN) was utilised. The group CRGV+AKI was BUN, creatinine, urinary specific gravity, and urine output, when available.

For the coagulation profile, which measured prothrombin time and aPTT, the QuickVet® PT/aPTT Coag Combo Test cartridge with the QuickVet® Analyzer was used.

AKI grade was given according to the IRIS Guidelines for acute renal injury (IRIS, 2016), based on the serum creatinine concentration (Grade I <140 µmol/L; Grade II 141 – 220 µmol/L; Grade III 221 – 439 µmol/L; Grade IV 440 – 880 µmol/L; Grade V >880 µmol/L).

The definitive diagnostic of CRGV in these cases was not performed, therefore, these cases are only suspected of being affected with this illness. Only two dogs that were euthanised were examined post-mortem in referral centres and diagnosed with TMA (major histopathological finding in CRGV) but this procedure was not performed in the emergency clinics, therefore is not reported in this thesis.

2.4 Imaging

For this study, when AKI was present, an AFAST ultrasound was performed to assess kidneys and bladder. For kidneys we can assess their size and recognize diffuse changes in echogenicity consistent with diffuse parenchymal disease, focal parenchymal changes such as kidney cysts and masses, kidney stones (nephroliths), renal infarcts, pyelectasia, hydronephrosis/hydroureter, retroperitoneal and perirenal (subcapsular) fluid and determine the presence of renal agenesis to exclude other causes of AKI (Lisciandro, 2014a).

For the assessment of the bladder, it is helpful to recognise urinary bladder wall abnormalities such as cystitis, polyps, and tumours. As well as that urinary bladder luminal abnormalities such as bladder stones (calculi), sediment, blood clots (Lisciandro, 2014b) and assessment of UOP (mL/kg/h) will need to be acknowledged in order to determine whether a dog or cat is producing acceptable volumes of urine. A urinary catheter is beneficial, but some animals can require sedation for this procedure which carries risk especially in the critically or acutely ill patient (Lisciandro & Fosgate, 2017).

2.5 Medical management

For patients presenting with skin lesions without AKI, the protocol used was to perform a minimum date base and according to those parameters institute the right plan. Urea was used to assess kidney function and determinate if the patient was at risk of AKI and if needed intensive treatment. For patients with systemic signs and AKI, there was no protocol instituted and treatment plan was up to the veterinary surgeon in charge of the case.

2.6 Statistical analysis

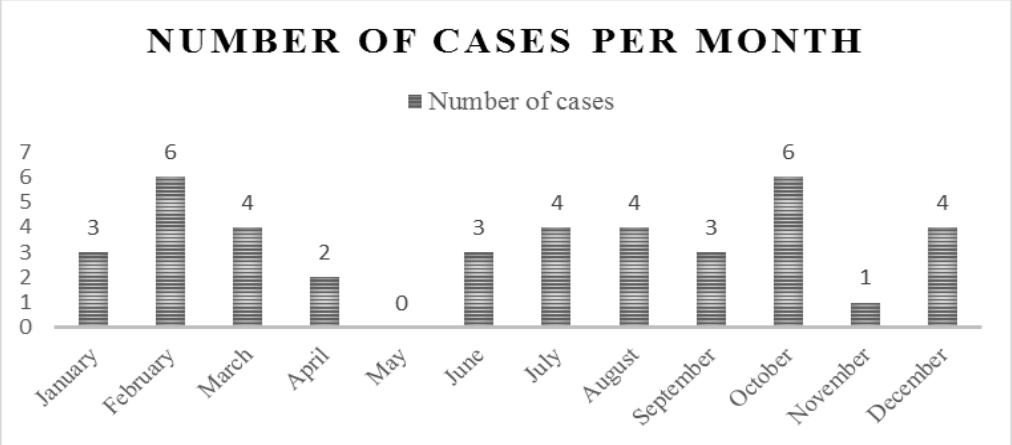
Data were analysed using Microsoft® Excel 2016 for Windows. Descriptive statistics were derived for all variables for both groups studied using the program IBM® SPSS® for Windows (version 20). The Shapiro-Wilk test was used to assess the normality of the data. Categorical variables were presented as proportions and/or percentages. Quantitative variables were expressed using mean and standard deviation when normally distributed and median and interquartile range when not normally distributed. For qualitative variables, frequency analysis was performed with the $\chi 2$ or Fisher exact test, when appropriate. To assess the difference between two independent groups when one of the variables is ordinal or continuous and not normally distributed the Mann-Whitney U Test was utilised. Qualitative data are expressed in absolute frequency and in percentage, quantitative data are expressed in means and standard deviation. Values of $p < 0.05$ were considered significant.

3. Results

3.1 Signalment

This study comprised 40 cases observed between 29th April 2015 and 29th April 2019. Cases are distributed per month in Graphic 1. These cases were divided into two groups: 13 dogs (32.5%) presented with skin lesions and later developed acute renal injury (group CRGV+AKI) and 27 dogs (67.5%) presented only with skin lesions (group CRGV-SL).

Graphic 1 – Number of cases between 29th April 2015 and 29th April 2019 (n=40) divided per month (original).



This study used data collected in 26 clinics from VetsNow group. Their location and number of cases were: Alfreton (n=1), Barton-le-Clay (n=2), Bournemouth (n=3), Bristol (n=1), Canterbury (n=1), Caterham (n=2), Coventry (n=1), Derby (n=1), Eastbourne (n=1), Edinburgh (n=1), Farnham (n=1), Guildford (n=1), High Wycombe (n=1), Lincoln (n=3), Macclesfield (n=3), Middlesbrough (n=2), Nottingham (n=2), Reading (n=1), Salisbury (n=3), Southampton (n=2), Stoke (n=1), Telford (n=2), Thamesmead (n=1), Warrington (n=1), Winchester (n=1) and Wrexham (n=1) (Figure 15). Breeds included were eight Labrador Retriever (20%), four English Springer Spaniel (10%), three Cocker Spaniel (7.5%), two German shepherd (5%) and one of each (2.5%) Flat-coated Retriever, Golden Retriever, Hungarian Vizsla, Siberian Husky, Jack Russel, Labradoodle, Rhodesian Ridgeback, Saluki, Soft-coated Wheaten, Staffordshire Bull Terrier and Water Dog. The remaining 30% of the dogs were crossbreed (n=13).

The Kennel Club (KC) group most represented were Gundogs (45%). This group includes the Labrador Retriever, English Springer Spaniel, Cocker Spaniel, Flat-coated Retriever, Golden Retriever, and the Hungarian vizsla. The following groups are Terrier (7.5%), includes Jack Russel Terrier and the Soft-coated Wheaten Terrier. The Hounds (Rhodesian Ridgeback and Saluki), Pastoral (German Shepherd) and Working (Siberian Huski and Water dog) represent 5% each.

From this group of breeds, 70% had their vaccines up to date (n=28), 7.5% were unvaccinated (n=3) and 22.5% (n=9) were not known to be vaccinated.

Age was not normally distributed; however, weight was. The median age at the time of the consults was 4.50 years, IQR ± 5 years (range 1 to 12 years old) and the mean body weight was 25.42 kilograms s.d. ± 8.86 kg (range 8 to 44 kilograms). 18 dogs were female (45%), 33% of these were neutered (n=6) and 77% were not (n=12); and 22 were males (55%), 73% neutered (n=16) and 37% were not castrated (n=6). In total, 22 animals were neutered (55%) whilst 18 were not (45%). The sex was not significantly associated with the risk of developing AKI (p = 0.435), neither the neuter status (p = 0.435).

Figure 15 – Map of cases across the United Kingdom (original).

Caption – Blue pin: one case; green pin – two cases; red pin – three cases.

3.2 Clinical History

The median time between the owners noticing the skin lesions and the consultation by the veterinary practices was 3.5 days (range 1 to 12 days). In the group CRGV-SL the median days between the owners noticing any skin lesions and going to their veterinary emergency service was 3.19 days (range 1 to 12 days), while the group CRGV+AKI showed similar data, 3 days (range from one to eight days).

Four animals from the group CRGV-SL were already being monitored for CRGV by their primary practice (wound management and renal parameters every day). One of these patients was prescribed metronidazole and corticosteroids for the skin lesions, however, no improvements were seen since the treatment began. Another patient had a recent history of skin

lesions, approximately for a week before presentation. This dog, therefore, had several blood tests performed at its daytime vets to monitor renal parameters, eight days before presentation. All have been normal, and skin lesions were healing.

In contrast, the group CRGV+AKI, five dogs (38.5%) were being treated already by their own practices for skin problems, all receiving different medication. 38.5% were prescribed NSAIDS (n=5), corticosteroids (n=1; 7.7%), amoxicillin (n=1; 7.7%), amoxicillin-clavulanate (n=2; 15.4%), metronidazole (n=1; 7.7%) and chlorphenamine (n=1; 7.7%). Three animals (23.1%) had their skin lesions cleaned with chlorhexidine and dressed.

Certain owners reported important information concerning where they walked their animals. Animals in the group CRGV+AKI were reported to have walked in Scotland (n=1), France (n=1), New Forest covering the area of southwest Hampshire and southeast Wiltshire (n=2), canal Llangollen, a navigable canal crossing the border between England and Wales (n=1), Rowney Warren Wood in central Bedfordshire (n=1) and within a close distance to the Kenley aerodrome in Surrey (n=1). In the other group, one dog swam in Combs reservoir, in Derbyshire, and three patients walked in Wales (in an area reported to have CRGV cases), Dale Lake and New Forest. Two dogs of the same household, after a walk in the woods, got the same skin lesions and presented at the clinics that exact same day, however, one of them developed AKI after and the other did not show any systemic signs.

3.3 Physical examination findings

Clinical presentations in the two groups were especially distinct. 19 dogs from the group CRGV-SL were bright, alert and responsive, although 13 were lethargic, and "*not themselves*" according to the owners. No other abnormalities were reported in the clinical exam with all vital signs within normal ranges. Urine output in this group, reported by the owners was within normal quantities. Dogs in the CRGV+AKI group differ on the physical examinations. Most common findings were tachypnoea (n=4), tachycardia (n=2), pyrexia (n=2), and hypothermia (n=2). Capillary refill time was increased in two animals, and pulse quality was frequently good (77%), however 3 animals had weak pulses. Mucous membranes were pink (n=9), brick red (n=2) or pale (n=2). 3 patients had a normal urine output, 5 were oliguric and 5 were anuric (based on the owner's clinical history). The systemic signs more commonly found were lethargy (n=10; 76.9%); vomiting (n=8; 61.5%), one of these had hematemesis; anorexia (n=6; 46.2%); diarrhoea (n=4; 30.8%), one was haemorrhagic; four animals were flat at presentation (30.8%). Vomiting was statistically significant with the development of AKI (p=0.034), although having diarrhoea did not to prove to have an association with this diagnosis (p = 0.075).

3.4 Skin lesions

Skin lesions in the group CRGV-SL were encountered in 14.8% of the animals in three different locations of the body (n=4), 51.9% in two locations (n=14) and 33.3% had skin lesions in one location only (n=9). More frequently, these lesions were found on the limbs (n=19), paws (n=11), face (includes mouth, nose, and cheeks; n=6) abdominal region (n=5) and eye (n=3). Less frequently were found in the thoracic region, genitalia, and ears (n=1). These wounds caused pruritus in 33.3% (n=9) of the dogs (animals presented signs such as scratching the wounds and licking them) and 59.3% presented signs identified as pain attributed to these cutaneous injuries (lameness, lethargy, pain when manipulated). All the 13 dogs who developed AKI (CRGV+AKI) presented with skin lesions in 1 location of the body only (n=8), 2 locations (n=2), three (n=2) and one patient had skin lesions in 5 different locations. Most frequently they were encountered in the paws, including digits, pads and interdigital skin (n=7), limbs (n=5), abdomen (n=4), eye painful or swollen and discharge (n=4), thoracically region (n=3), mouth (n=2) and head (n=2). Only 2 animals showed signs of pruritic skin lesions and all the thirteen patients presented signs of pain.

The macroscopic aspect of the skin lesions reported by the veterinarians varied, within and between the two groups. In the group with only skin lesions, CRGV-SL, one animal had lesion with 0.2 cm diameter (3.7%), eight with 0.5 cm (29.6 %), nine animals with 1 cm (33.3%) and nine animals with 2 cm (33.3%). Serous discharge was present in six animals (22.2%), inflammation characterised by erythema, pain and swelling were present in 21 animals (77.8%), purulent discharge present in one dog (3.7%), dermatitis (n=3; 11.1%). Most commonly superficial abrasions were described (n=17; 63%) and cutaneous ulcers (n=9; 33.3%).

One dog had an infected ulcerated lesion on his nose, this was caused by the dog consistently scratching the wound. The distal nose just caudal to nasal planum was raised and ulcerated. One dog had lesions on his genitalia presented with lesions like lumps, ulcers in all his limbs, around his nails and pads.

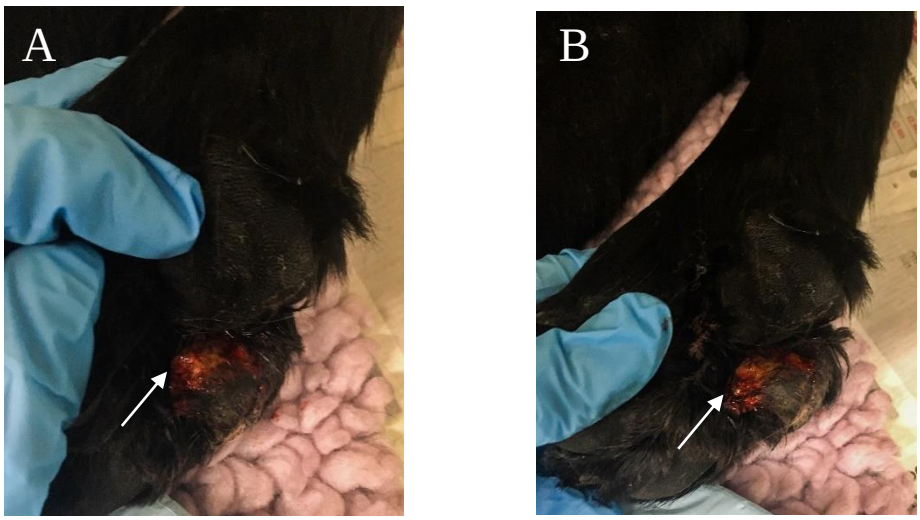
Macroscopic description of the lesions in group CRGV+AKI varied in size: 0.2 cm (n=2; 15.4%), 0.5 cm (n=2; 15.4%), 1 cm (n=3; 23.1%), 2 cm (n=1; 7.7%), 3 cm (n=2; 15.4%) and 5 cm (n=3; 23.1%). Three animals had serous discharge, three had exudative discharge and one had purulent discharge. Description of these lesions were described as superficial abrasions (n=5; 38.5%), cutaneous ulcers (n=7; 53.8 %) (Figure 16, A and B), dermatitis (n=3; 23.1%), and pustules and papules (n=2; 15.4%).

Common features encountered were inflammation, along with erythema, pain and swelling (n=10; 76.9%), alopecia (n=3; 23.1%) and haemorrhage (n=1; 7.7%). One dog had all nails

affected and these did not appear to be growing properly and presented an inflammatory discharge and hair loss. One patient developed oedema of the pinnae and muzzle and another had necrotic lesions on the limbs and feet.

The number of locations of different skin lesions did not prove to be correlated with a worst prognosis (p=0.821), however, the size of the skin lesions correlated positively with the AKI diagnosis. Lesions wider than five centimetres were significantly correlated with the development of AKI (p = 0.029).

Figure 16, A and B – Skin lesions in a dog confirmed with CRGV, from Macclesfield. Ulcerative wound in the ventral aspect of the right hind, affecting the pad and skin (arrow) (photos taken by Lesley Moore, RVN).



3.5 Diagnostics

CRGV is reported to cause acute renal injury so it was advised to all owners to check for dehydration, blood glucose level, tissue perfusion, and a kidney parameter to monitor the treatment plan. Animals from the group CRGV-SL did not present systemic signs (except for lethargy; n=13), so it was advised to do only the minimum data base (MDB). Urea was the parameter utilised to assess renal function in this group. Median blood urea nitrogen (BUN) was 8 mmol/L IQR ± 6 mmol/L.

In the group CRGV+AKI due to the systemic signs, further tests were required. MDB and EPOC® were performed in 11/13 cases. The median results were within normal parameters except for the kidneys values which were increased (BUN and creatinine). Median BUN was 27.5 mmol/L IQR ± 29.5 mmol/L and median creatinine was 205 mmol/L IQR ± 400 mmol/L.

Table 1 – EPOC® results (original).

EPOC®	Number of cases	Median	Minimum and maximum	Reference range
pH	n=11	7.405	7.270 - 7.476	7.351 - 7.443
pCO_2 (mmHg)	n=10	33.5	24.0 - 41.0	33.6 - 41.2
HCO^{3-} (mmol/L)	n=10	21.2	15.1 – 24.0	20.8 - 24.2
NA^{+} (mmol/L)	n=11	144	114 – 149	139 – 150
K^{+} (mmol/L)	n=11	4.2	3.7 – 5.7	3.4 - 4.9
Ca^{++} (mmol/L)	n=11	1.09	0.56 – 1.34	1.12 - 1.40
Cl^{-} (mmol/L)	n=11	111	96 – 119	106 - 127
Glucose (mmol/L)	n=11	6.4	4.5 – 34.6	3.3 - 6.4
Lactate (mmol/L)	n=11	1.29	0.7 – 4.5	0.60 - 2.90
Creatinine (mmol/L)	**n=11**	**205**	**122 - 1326**	**44 - 115**

The median of the ionized calcium (Ca^{++}) was lower than expected (median 1.09 mmol/L IQR ± 0.38 mmol/L). Although median PCV and TS were within normal values, four dogs had an increase in PCV parameter, and therefore were dehydrated. Two animals had a low PCV confirming anemia pre- or non-regenerative.

One dog had a blood glucose of 35.8 mmol/L (reference range between 3.3 – 6.4 mmol/L), but the cause of this was unknown. The urine dipstick did not show glucosuria, no PU/PD, weight loss or other systemic signs and after neutral insulin, the values were within normal range.

RapidBac™Vet was performed in one dog that tested positive for bacteriuria. Urinalysis was likewise executed in five dogs. The results were proteinuria (n=5), hematuria (n=2), hemoglobinuria (n=1) and the pH was sited between 6.5 - 8.5. The urine specific gravity was measured in five dogs, using a refractometer demonstrating a mean value of 1.018 (range 1.013 to 1.026; reference interval > 1.035).

A blood smear was evaluated in five animals in the CRGV+AKI group. Thrombocytopenia (n=2) was observed, neutrophilia with a left shift (n=2) and monocytosis (n=1). Anisocytosis or poikilocytosis was not observed.

Concerning the coagulation profile, PT and aPTT were calculated in two dogs. PT was 20.3 seconds and 17 seconds (reference range 14 – 19 seconds), and aPTT was 115.2 seconds and 93 seconds (reference range 75 – 100).

P.O.C.U.S abdominal ultrasound was performed in five patients however no significant abnormalities were encountered.

AKI grade based on the IRIS guidelines, based on the creatinine level were six dogs in grade I (46.2 %), two in grade II (15.4%), one in grade III (7.7%), one in grade IV (7.7%) and three animals in grade V (23.1%).

Table 2 – Biochemistry results in three dogs with AKI (original).

Parameter	dog 1	dog 5	dog 6	Mean	Reference range
Total protein (g/l)	54	52	70	59	52 - 82
Albumin (g/l)	25	19	34	26	22 - 39
Globulin (g/l)	29	33	36	33	25 - 45
ALT (u/L)	>1000	266	83	>450	10 - 100
ALKP (u/L)	1067	174	56	432	23 - 212
Total bilirubin µmol/L	186	67	7	87	0 - 15
Amylase (u/L)	1145	2013	479	1212	500 - 1500
Lipase (u/L)	1450	2137	566	1384	200 - 1800
Creatinine mmol/l	>1000	913	157	690	44 - 159
Urea mmol/l	40.5	36	23.7	33.4	2.5 - 9.6

Biochemistry results were obtained from three dogs (Table 2). All patients had azotaemia (elevated creatinine and urea). Dog number 6 did not present with any further abnormalities. Dogs 1 and 5 presented with hyperbilirubinaemia and elevated liver enzymes (ALT and ALKP). Dog 5 had hypoalbuminaemia, increased amylase and lipase.

3.6 Medical management

Based on the physical examination findings and laboratory results, the veterinary surgeon in charge of the case chose the most adequate plan for each patient. The group CRGV-SL only received treatment regarding skin lesions. 13 patients had the wound region clipped and cleaned with chlorhexidine (48%). In seven patients a cream containing betamethasone and fusidic acid was applied to the wound. One patient received dexamethasone (0.01 mg/kg i.m. q24 hours) for management of the lesions. Long-action antimicrobials were administered to 13 patients: two received amoxicillin (7 mg/kg i.m. q24 hours) and 11 amoxicillin-clavulanate (8.75 mg/kg i.m. q24 hours). Pain management was assessed as well. Buprenorphine (0.02 mg/kg i.m. q6 hours) was administered to three patients and one patient has prescribed Tramadol (2-5 mg/kg p.o. q24 hours). Two patients that had an increased BUN received intravenous fluid therapy, at a rate of 2 ml/kg/hour (7.4%). Eight patients did not receive any type of treatment.

The animals where AKI was confirmed were admitted to the clinics and received medical treatment due to their deteriorated state or their clinical laboratory findings (n=12), except for one patient due to financial restraints. Fluid therapy was an important component of the

treatment plan. The animals that presented with signs of shock (n=6) received a bolus of 20 ml/kg and then a constant rate was instituted. Six animals were on 6 ml/kg/hr (46.2 %), three on 8 ml/kg/hr (23.1%) and one of each (7.7%) on 5, 6 and 10 ml/kg/hr. The fluid type selected for the 12 patients was Hartmann's solution.

Antibiotics prescribed were cefuroxime (n=3), amoxicillin-clavulanate (n=2) and metronidazole (n=1). The remaining patients did not receive any antimicrobials.

Regarding wound management, six animals (46.2%) had their wounds cleaned with chlorhexidine diluted with water and dressed, and a silver sulfadiazine cream was applied to 3 animals (23.1%). An antihistamine, chlorpheniramine, was administered to three patients (23.1%) and dexamethasone to four (30.8%).

CRGV requires pain management, consequently, buprenorphine was administered to six dogs (46.2 %), methadone (n=4; 30.8%) and fentanyl CRI in one patient (7.7%).

For management of gastrointestinal signs, a proton-pump inhibitor was selected in four patients (30.8%), maropitant for vomiting and visceral pain (n=2; 15.4 %) and metoclopramide (n=1; 7.7%). Furosemide was administered in one patient (7.7%) for oliguria.

3.7 Outcome

CRGV-SL group was advised by the emergency veterinary surgeons to monitor their pet at home and be vigilant about their condition. Monitoring included being attentive to any type of deterioration in the patient's mental status, wounds that would not improve or infected and urine output. No patient within this group was reported to have returned to the clinics for skin lesions or development of AKI. Six patients (22.2%) were discharged normally without any medication or advised to see their own veterinary practices. 19 patients were advised to visit their own clinics the following day to re-check the wounds and assess the therapy instituted. The therapy prescribed was antibiotics (amoxicillin-clavulanate tablets to 5 patients) and NSAIDS, particularly meloxicam to six dogs (0.1 mg/kg p.o. q24h) and firocoxib to one dog (5 mg/kg p.o. q24h). A buster collar was advised to all patients to prevent wound licking and further injuring.

Follow up exams, within the CRGV+AKI group, consisted of repeating the MDB and EPOC 6-12 hours after initiating the treatment. These exams were performed in six animals (n=6). Most common complications seen were azotaemia (n=4; 30.8%), hypocalcaemia (n=2; 15.4%), hyponatraemia (n=2; 15.4%), hypochloraemia (n=1; 7.7%) and hyperkalaemia (n=1; 7.7%).

Urine output was recorded at discharge in eight patients (urinary catheter) and three animals were anuric, two were oliguric, two normal urine production and one was polyuric.

Mean days since these animals were admitted until discharge or euthanasia was five days (range

two to ten days). Five animals were submitted to euthanasia due to poor quality of life (38.5%) at owner's request, after they remained oliguric and the follow-up exams did not show any improvements, particularly the kidney parameters (azotaemia was still present after fluid therapy). Two animals (15.4%) went home after the consult due to financial constraints and did not return to the clinic. Seven animals (53.8%) were discharged to their own practices for continuing or changing the treatment. No animals from this group returned to the clinics.

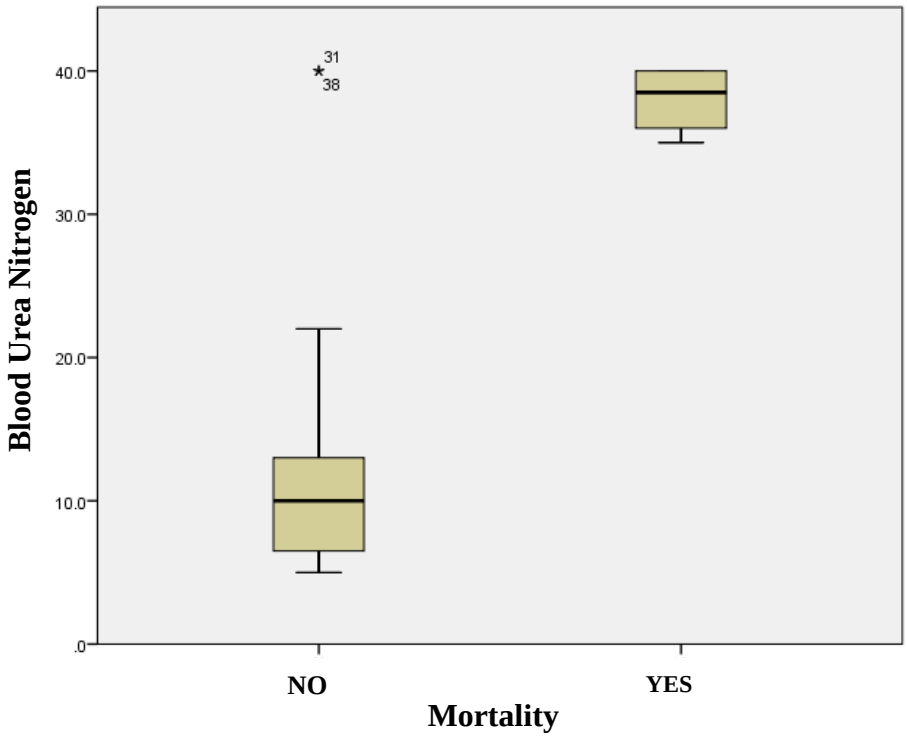
An association between clinical signs and a worse outcome (death) were reviewed.

Vomiting and the presence of AKI proved to have a positive correlation with the worst outcome ($p = 0.015$ and $p=0.008$, respectively). Diarrhoea was, likewise, proved to be statistically associated with the worse outcome ($p = 0.010$). These results reinforce the theory that dogs who develop AKI due to CRGV are more likely to die or be euthanised.

Other prognostic indicators were also calculated. Hypocalcaemia ($p= 0.003$), oliguria or anuria ($p<0.001$), and hypophosphatemia ($p<0.001$) were proven to be very significantly correlated with a worse outcome (death). Graphic 2 represents the median BUN in the group of dogs that died (yes) and the group of dogs that survived (no).

Histopathology was not performed by any of the emergency clinics, but two referral centres did the post-mortem exam in two dogs that were euthanized by the emergency practices. This confirmed the TMA present in CRGV.

Graphic 2- Boxplot comparing the median BUN between two groups of dogs with CRGV. Group 'Yes' represents the dogs that died and Group 'No' the dogs who survived.



4. Discussion

Cutaneous and renal glomerular vasculopathy is a possible fatal disease of unknown aetiology. This retrospective study describes 40 cases diagnosed across the UK, contributing to a better knowledge of the disease and sensitizing the clinician for this uncommon and poorly understood disease. In the mid-1980s, it was only reported to occur in six months to six years of age racing and training greyhounds, with no sex predilection. It affects only the skin and kidneys (Carpenter, et. al, 1988; Hertzke, et. al, 1995). However recent studies, including this thesis, have demonstrated that it can affect a variety of breeds, encompassing all ages and not only young adults (Holm et al., 2015).

The first study to provide signalment risk factors for CRGV in dogs in the UK was performed in 2018. It proved that breed, KC breed group, neuter status, and sex were significantly associated with the occurrence of CRGV, yet age was not a significant risk factor. This study compared n=101 dogs with confirmed CRGV to a denominator population of 446,453 dogs (Stevens et al., 2018). In this study, female dogs were significantly more likely to be diagnosed with CRGV than males (Stevens et al., 2018). The gender in this thesis was not consistent with this previous report, as more males were diagnosed. This fact can be explained by the small sample size (n=40 vs n=101). In fact, gender predisposition is still unclear (Stevens et al., 2018). Some TMA's in humans, particularly TTP, are reported to occur more frequently in women (Shatzel & Taylor, 2017). Similarly, female dogs are more prone to canine idiopathic thrombocytopenic purpura than male dogs (Lewis & Meyers, 1996). Further studies are needed to better clarify this predisposition.

Also, neutered status did prove to be consistent, as more animals that were neutered were identified with CRGV. The odds of neutered dogs being identified with CRGV was 3.36 times that of entire dogs (Stevens et al., 2018).

Two KC breed groups, gundogs, and hounds are between nine and ten times more likely to be diagnosed with CRGV than terriers, while no toy dogs were diagnosed with the disease (Stevens et al., 2018). These facts agree with this study, as Gundogs were the most represented group (45%). An explanation for this relies on the fact that this group includes the three most popular breeds in the UK: Labrador retriever, Cocker spaniel, and English Springer Spaniel (The Telegraph, 2019). According to the KC study, are more prevalent in areas with an elevated risk of CRGV occurrence. Even though this disease has been reported from various locations across the UK, breed popularity differs throughout the country. The study by the UK KC analysed the number of dog registrations by breed in ten UK regions in 2016, suggested that different regions each have their top ten preferred breeds. Gun dogs and hounds may prevail in rural areas where

owners may participate in countryside sports such as shooting and hunting in geographical areas where may occur more commonly a risk of CRGV occurrence (Stevens et al., 2018).

These three breeds were also the most represented in this study (20%, 10%, and 7.5%, respectively). Toy breeds were not diagnosed with the disease in previous reports, including this one. In the study conducted by Stevens (2018), breeds showing increased odds of CRGV compared with crossbreds included Hungarian vizslas, flat-coated retrievers, whippets, and English springer spaniels. Breeds with reduced odds encompassed German shepherd dogs, Jack Russell terriers, and Staffordshire bull terriers. In this study, two German shepherds and one Jack Russel and Staffordshire bull terrier were amongst the breeds. It would have been interesting to see the odds ratios from this dissertation and compare it with a data base of dogs in a population, but the lack of this data constitutes a limitation for this study.

Age and weight of the population of this study were consistent with previous reports of the similar spectrum (Holm et al., 2015; Skulberg et al., 2018; Stevens et al., 2018). The majority of the animals were young adults, but the range is very wide (1 to 12 years old). Only two patients weighed less than 15 kilograms, which may lead to the suspicion that this disease affects more commonly large breeds (>20 kilograms).

CRGV cases are reported generally between November and May (winter/spring) with a third of all cases diagnosed in the first three months of the year (January to March) (Stevens et al., 2018). In this thesis, 50% of cases were reported in this period, with n=13 cases in January, February, and March (32.5%), however, cases confirmed throughout the year were constant, except in May where no cases were reported over the four year period. This contrasts with human STEC-HUS, where most cases occur in summer or autumn months (Bruyand et al., 2018).

These changes might be due to the possible climatic drivers of this disease. Minimum and maximum temperatures during the colder months in the UK are increasing. This may potentiate good environmental conditions for an evolving organism or a novel ecological niche for a pathogen that had been current in the environment, but could not evolve in the previous conditions (Stevens et al., 2018). Although, these climate changes alter the occurrence of CRGV, in 2019 there have been less frequent cases confirmed in the three first months of the year compared to 2018, but an explanation for it is still unclear. The association between increased case numbers and milder, wetter weather in winter and spring is being studied and assessed in order to find a reason (Woodmansey, 2019).

When CRGV first appeared in the UK, in 2012, three cases were reported during this year. These cases were randomly distributed all over England, but in the ensuing years, there was a tendency to cluster in specific areas, more specifically two clusters: New Forest and

Manchester. North-east of England and the New Forest region of south England have the highest five-year density of CRGV cases (Stevens et al., 2018). This is the same as Haemolytic uremic syndrome due to Shiga toxin-producing Escherichia coli infection, which is reported to occur in well-identified clusters (Bruyand et al., 2018).

As we can observe from the clinic's location (see Figure 15), South-east region had the highest case number (n=11 cases; 9 clinics), confirming the prevalence of this disease in the area. Three dogs were reported by the owners to have walked in New Forest before the skin lesions appeared. The New forest consists mainly by broadleaved woodland, so is Rowney Warren Wood (near Barton-le-clay, East England). As demonstrated by Stevens, et. al (2018), habitat is known to have the highest relative contribution to CRGV incidence (20.3 %), particularly woodlands and lowland dry heath communities.

Four different manifestations that typify this illness was described by Carpenter in 1988: greyhounds who only developed ulcerative skin lesions (73.8%), dogs with systemic signs of illness and onset of azotemia along with skin lesions (17.8%), the ones who develop skin ulceration and develop azotaemic within ten days, and dogs who develop AKI (4.2%) and only after skin lesions, and survive with treatment (4.3%) (Carpenter et al., 1988). The percentage of cases in this study which developed only skin lesions was 67.5%. Compared to the cases who develop AKI, which was 32.5%, there is a bigger proportion of cases who do not develop AKI. It would have been noteworthy to review dermal and renal histopathology in these dogs. However, the lack of antemortem tests, and cutaneous biopsies, with dermal histopathology, only may raise concerns about the diagnosis and is not confirmative of the CRGV, therefore it is impossible to be conclusive. This is, undoubtedly, a limitation for this study, and further studies to identify the exact number of animals with only skin lesions due to CRGV are required, as the skin biopsies merely reveal nonspecific, ischemic changes, involving ulceration of the epidermis with coagulative necrosis of the subjacent dermis (Jepson et al., 2019). In patients that presented at the emergency clinics with skin lesions and without clinically confirmed AKI, we attributed these to CRGV, but it is impossible to confirm this association. However, from the clinical exam, the area where the animals live and walk, the wounds macroscopic presentation and the fact that the healing is slow can lead us to the diagnosis. The fact that we had two animals from the same household presenting skin lesions on the same location of the body and only one developed AKI, can show us that an environmental trigger could be correlated with this disease. It would have been interesting to see if these animals were the same breed or familiars, as this could have led us for a more genetic component of the disease.

Macroscopic presentation of the wounds in the two groups of animals (with and without AKI) was similar and correlated to previous reports (Carpenter et al., 1988; Hertzke et al., 1995; Holm et al., 2015). Limbs, face, and paws, including digits and interdigital skin, were the most frequent locations where the wounds were observed. Tongue wounds were less frequent (4%) in previous reports, and in this dissertation were not reported (Jepson et al., 2019). In this study, lesions ranged from small ulcerative lesions to large ulcerated round lesions to central necrosis on the limbs. Occasionally their primary practices described as a purulent pyoderma, pododermatitis, or an allergic reaction.

In almost every wound alopecia, erythema, swelling, and oedema, especially when located in the limbs and digits were described. These wounds progressed fast (maximum 24 hours), and the ones being treated before were not improving as expected (n=4). However, one dog was already being monitored for CRGV at his primary practice (kidney parameters daily) with normal results, and his wounds were healing. AKI usually develops in a mean of 4 days (1-9 days) (Holm et al., 2015) therefore this dog may have had skin lesions without AKI, and the risk of developing kidney injury decreased. Monitoring for AKI when skin lesions appear must be continued up to ten days after the cutaneous problems, daily (Holm & Walker, 2018). Meantime from development of skin lesions to diagnosis of AKI was particularly similar to previous reports (Carpenter et al., 1988; Holm et al., 2015; Holm & Walker, 2018). It was interesting to notice that lesions wider than 5 cm were positively correlated with the diagnosis of AKI. Further studies to describe the size of the lesions and the AKI due to CRGV are of the utmost importance, as it can help clarify exactly which animals are more likely to develop AKI based on their wound size and macroscopic aspect.

Dogs diagnosed with CRGV are initially presented to veterinarians for assessment of these skin lesions, therefore some animals had been seen by their own veterinary practices. One dog was prescribed steroids, however, there is currently no evidence that the use of this drug at immunosuppressive or anti-inflammatory doses is beneficial (Holm & Walker, 2018). Immunosuppressive therapy was administered ineffectively to one Great Dane in Germany (Rotermund et al., 2002), nonetheless, guidelines for human TMAs recommend that adjuvant corticosteroid therapy must be given to all patients (Allford, Hunt, Rose, & Machin, 2003).

Another animal was prescribed metronidazole for skin lesions, however for skin problems this antimicrobial is not appropriate or the first line of treatment. First-line antibiotics consist on well-established and tolerated broad-spectrum drugs with anti- staphylococcal activity (Beco et al., 2013) as the vast majority of skin infections in companion animals are associated with coagulase-positive staphylococci, with *Staphylococcus pseudintermedius* the most common causative agent in canine pyoderma (Devriese et al., 2005).

Renal function was assessed through urea. All the results were within normal levels, except for this parameter which was mildly elevated. Blood urea nitrogen (BUN) and creatinine may be raised, but AKI should not be ruled out if azotemia is not present (Ross, 2011). Even though some animals had their urea raised, none of them were admitted to the hospital, as their clinical signs and other parameters were within normal ranges. However, 19 patients were advised to visit their own veterinary practices to re-assess the wounds and monitor the kidney function. Previous reports reveal that non azotaemic dogs with CRGV tend to have reduced glomerular filtration rates and renal histopathology showing mild, multifocal, endothelial glomerular changes (Holm et al., 2015). If initial investigations confirm that there is no evidence for AKI then appropriate monitoring of renal function and lesion management is all that is required (Holm & Walker, 2018). Initial laboratory evaluation should include a complete blood count, serum biochemistry profile, assessment of acid-base status, urinalysis and urine specific gravity (Ross, 2011). Henceforth, this constitutes a limitation in this study, as we were unable to perform additional tests and evaluate with accuracy the kidney function of the group animals with skin lesions only. When at risk for CRGV more tests are required to completely evaluate the kidney and calculate the risk that these animals are exposed to develop AKI.

Some of these patients were prescribed antibiotics for the skin lesions and NSAIDs, meloxicam or firocoxib. NSAIDs that do not target COX-1 activity have demonstrated less gastrointestinal toxicity, but no NSAIDs has been proven to be safe for the kidney. The kidney is the organ with the second-highest reports of adverse drug events which typically manifest as functional changes (Lomas & Grauer, 2015). In CRGV, where the risk of developing AKI is elevated, within ten days of developing skin lesions, the NSAIDs are contra-indicated (Holm & Walker, 2018). In the group of 13 dogs with AKI, 38.5% received NSAIDS by their primary practice for the skin lesions. Still, in one report, histopathologic lesions in the kidneys were not attributed to NSAIDS nephrotoxicity, however, they may have exacerbated these lesions (Holm et al., 2015).

Most common clinicopathological findings observed in this case series in dogs with AKI were azotaemia, anaemia (pre- or non-regenerative), proteinuria, haematuria, poorly concentrated urine, hypocalcaemia, thrombocytopenia and neutrophilia, high serum liver enzyme activity, and hyperbilirubinaemia, similar to abnormalities previously reported (Carpenter et al.; Skulberg et al., 2018). Hypoalbuminaemia was identified only in one dog which contrasts with previous reports. 63.3 percent of the confirmed CRGV cases had decreased albumin (Holm et al., 2015). It is important to differentiate this disease from Leptospirosis as the clinical and laboratory abnormalities are especially alike (Miotto et al., 2018). In the study conducted by Holm (2015), leptospiral organisms were not identified in serology, PCR on peripheral blood,

renal and hepatic tissue, and histopathology was not compatible with leptospirosis. Histological changes in the kidneys of dogs affected by leptospirosis are subtle, and included mild renal tubular simplification, with single-cell necrosis and attenuation, along with minimal interstitial lymphoplasmacytic inflammation, oedema, and haemorrhage. Hepatic lesions consist of scattered hepatocellular single-cell necrosis and hepatocellular dissociation (Rissi & Brown, 2014). In CRGV there are no typical hepatic lesions and the predominant renal lesion is TMA (Hertzke et al., 1995; Holm et al., 2015). In addition, skin lesions were not previously reported along with leptospirosis infection, except for calcinosis cutis in young dogs (Munday, Bergen, & Roe, 2005), which was not identified in CRGV in other reports, including this one (Holm et al., 2015). Haematology, when leptospirosis occurs, will regularly reveal leucocytosis and thrombocytopenia. When coagulation is compromised, secondary regenerative anaemia can occur caused by bleeding or disseminated intravascular coagulopathy (DIC) (Maele, Claus, Haesebrouck, & Daminet, 2008). DIC occurs in 18.4 % of animals with leptospirosis (Schuller et al., 2015). Coagulation times in dogs with CRGV are not consistent with DIC and there were no clinical signs suggesting this illness (Gando, Levi, & Toh, 2016).

Another feature observed in this study was ionized hypocalcaemia. This parameter is a prognostic indicator and when present it can disclose a more difficult to recover from AKI, along with severe azotaemia, oliguria/anuria, and proteinuria. Dogs that present with these clinical features are less likely to survive AKI (Vaden, Levine, & Breitschwerdt, 1997). Two dogs in this study failed to improve ionized calcium levels. One was euthanised and the other was discharged to his member practice but follow up information is not available.

Elevated liver enzymes have been reported before, and also in this study (Holm et al., 2015; Skulberg et al., 2018). Hyperbilirubinemia might be due to the haemolytic anaemia present in this disease. One important complication in AKI that was not assessed in this case series is hypertension. The median blood pressure in dogs with CRGV was 176 mm Hg (range 102–280 mm Hg) at the time of onset of AKI (Holm et al., 2015).

CRGV is a disease with a high mortality rate when AKI is present (Carpenter et al., 1988). Treatment objectives are to limit further renal damage and improving cellular recovery by correcting fluid, electrolyte, and acid-base disorders, achieving and maintaining normotension, and establishing/maintaining urine production. Guidelines on the management of AKI are chosen to treat CRGV (Jepson et al., 2019). Some dogs that developed azotaemia and were IRIS grade I recovered after intensive fluid therapy and proper treatment. This suggests that CRGV is not a potentially fatal disease, but with the appropriate treatment for each patient, it can be reversible. The difficulty of confirming the diagnosis without renal biopsy made it problematic to know and choose the management of each case (Holm et al., 2015).

Differential diagnoses for canine TMA include CRGV (Carpenter et al., 1988) and HUS (Chantrey et al., 2002; Holloway et al., 1993; Orco et al., 2005). In Human Medicine, plasma exchange therapy is recommended in adults with STEC-HUS, as well as, antibiotic administration, monoclonal Shiga toxin antibodies and renal transplantation (Ruggenenti et al., 2001). Plasma therapy is the gold standard for the management of aHUS, however eculizumab, a recombinant humanized monoclonal antibody directed against C5 has been proven highly effective with approximately 85% of patients becoming disease-free (Kavanagh, Goodship, & Richards, 2013). This type of therapy is yet to be evaluated in CRGV, but due to the similarity between this illness and aHUS could be a starting point. The success of PEX therapy in dogs with CRGV in the two survivors (n=6), in light of the high mortality reported with CRGV, warrants further consideration whether PEX could prove helpful in selected cases (Skulberg et al., 2018).

Antiplatelet therapy seems like another potential therapeutic consideration, given the etiopathogenesis of CRGV. Aspirin and dipyridamole have both been used in the primary treatment of TTP. A 78% response rate at six months was achieved when aspirin and dipyridamole were administered in conjunction with plasma exchange. These guidelines recommend administrating a low-dose aspirin when platelet recovery is observed (Allford et al., 2003).

Although aHUS and CRGV share some similarities, skin lesions are very rare and therefore unrecognised as a specific symptom of aHUS (Ardissino et al., 2014). Histopathological lesions in these case reports were leukocytoclastic vasculitis, which is characterised by upper dermal perivascular infiltrates, primarily comprising neutrophils with karyorrhexis of nuclei and fibrinoid necrosis of vessel walls, similar to previous reports on CRGV (Hertzke et al., 1995). It is yet unknown if CRGV is a new canine disease or a variant of HUS or aHUS, HUS of unknown aetiology or TTP (Holm et al., 2015).

5. Conclusion

CRGV is a TMA of unknown aetiology which, when AKI develops, appears to carry a severe prognosis. CRGV is an emerging disease or, one that was previously present but unrecognised, in the United Kingdom. However, the cause and any link with an infectious agent remains uncertain (Holm et al., 2015). Vasculopathy, preferentially concerning the small vessels of the skin and kidneys in dogs, appears to be unique to CRGV and has not, to the authors' knowledge, been reported associated with any other canine disease.

CRGV occurs through outbreaks in a distinct seasonal pattern with more than 90 percent of cases reported between November and May while the area from which cases have been reported

has expanded since 2012 to encompass most of the western and southern regions of England. CRGV occurrence is most frequently associated with woodlands and lowland dry heath. Therefore, these factors together can raise awareness for local veterinarians and owners to be more sensitized for this disease. In addition, Gun dogs and hounds have an increased risk of developing CRGV in the UK, while terriers and toy dogs appear to be the breed groups least at risk. Specific breeds with increased odds of CRGV included Hungarian vizslas, flat-coated retrievers, whippets, and English springer spaniels. However, further studies investigating the distribution of specific breeds and breed groups in the UK would help to determine if they are indeed inherently more predisposed or whether the results are a result of an increased proportion of such breeds in areas of greater risk. A population of non-azotaemic dogs with CRGV exists, and for such cases, the prognosis may be good. Heretofore, there are no tests that can predict this number of dogs, that only develop skin lesions. This study proved that dogs presenting skin lesions larger than five centimetres have a greater chance of developing AKI and thus these dogs need monitoring, as the risk of AKI is higher. Further studies are required to understand the CRGV pathogenesis and its different manifestations. For the population of dogs that develop azotaemia and particularly oligoanuric AKI, the prognosis can be guarded. However, intensive medical therapy is indicated in these patients because full recovery from AKI was successful in seven animals, which agrees with previous studies. Continued detailed clinical, clinicopathological and epidemiological evaluation will further augment the understanding of the disease and will hopefully help to identify possible underlying infectious triggers and immune dysregulation, which have been related with similar TMA conditions in humans, define prognostic indicators and determine the most suitable management for these patients. CRGV remains a rare disease in the UK, however, it has the potential to be a life-threatening condition. Cases now appear to be widespreading across the UK and veterinarians need to be familiar with the history, clinical signs and diagnostic approach to these patients.

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