TITLE: Cutaneous and Renal Glomerular Vasculopathy: What Do We Know so Far?

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ARTICLE TITLE

Cutaneous and Renal Glomerular Vasculopoathy: What do we know so far?

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None relevant

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KEY POINTS

- Cutaneous renal glomerular vasculopathy (CRGV) is a condition resulting in thrombotic microangiopathy (TMA) recognized with apparently increasing prevalence in the United Kingdom since 2012.
- A causative infectious etiology has not been identified to date.
- Clinical progression of cases usually involves identification of ulcerated skin lesions, typically affecting the distal limb and progression to oligoanuric acute kidney injury. However, subclinical manifestations of this condition with less severely affected individuals are believed to occur.
- Mortality rates for dogs that develop oligoanuric acute renal failure is very high, although a limited number of dogs have been reported to survive with intensive supportive care.

SYNOPSIS

Cutaneous renal glomerular vasculopathy (CRGV) or colloquially named ‘Alabama rot’ is an emerging condition in the United Kingdom, previously reported from the USA and Germany. The etiology of CRGV is not yet determined and no definitive link to an infectious agent has been made. Dogs diagnosed with CRGV initially develop cutaneous lesions and a proportion of these dogs go on to manifest acute kidney injury which may result in oligoanuric acute renal failure. Ante-mortem diagnosis is challenging given the lack of a specific diagnostic test and confirmation of CRGV is therefore currently dependent on identification of thrombotic microangiopathy on renal histopathology. Further work is required to better understand the etiopathogenesis of this condition so that more specific diagnostic tests and interventions for treatment can be developed.

Title: Cutaneous Renal Glomerular Vasculopathy: What do we know to date?

Introduction

Cutaneous renal glomerular vasculopathy (CRGV) or the condition that has colloquially been termed 'Alabama rot' has been recognized with apparently increasing frequency in the United Kingdom (UK) since 2012. The etiopathogenesis of this condition remains incompletely understood. Despite speculation and preliminary infectious disease testing of confirmed cases, an infectious etiology has not been identified. However, the chronology of presentation, diagnostic testing, and small sample size are limiting factors. The condition has gained much attention due to the high mortality rate reported in dogs that develop oligoanuric acute renal failure associated with CRGV. Lack of a definitive ante-mortem diagnostic test and reliance on renal histopathology for confirmation of this condition, means that the true prevalence and incidence of CRGV within the canine population remains unknown. Continued work is required to better understand the epidemiology of CRGV and to explore individual, genetic, environmental or infectious associations that underpin the risk of dogs developing CRGV. Ultimately it is likely that only through a better understanding of the pathogenesis of CRGV, that diagnostic tests can be developed that may facilitate early diagnosis and intervention that improves survival.

History of CRGV and 'Alabama rot'

Conditions compatible with cutaneous renal glomerular vasculopathy were first reported in the veterinary literature in Greyhounds from the USA in the late 1980's.[1,2,3] These cases were variably associated with both skin lesions an acute kidney injury (AKI) and an underlying etiology was not identified. Since 2012 a form of CRGV has been recognised in the UK. It is unclear whether the etiology for these UK cases is the same as historical cases, but the clinical progression of cases and histopathological manifestations are similar. Dogs which develop CRGV commonly present with ulcerated skin lesions, with development of AKI a median of 4 days after skin lesions are noted.[4] The presence of skin lesions is rarely reported in dogs with other causes of AKI making the clinical presentation of dogs with CRGV unique. The common feature of all dogs with CRGV is the histopathological finding of thrombotic microangiopathy (TMA) in renal tissue. Differential diagnoses for TMA in dogs includes CRGV but also hemolytic uraemic syndrome (HUS). HUS has previously been reported in 5 dogs including a number of different breeds, such as the Yorkshire terrier, miniature poodle, Labrador retriever, German shepherd dog.[5,6] Of the HUS cases reported in the literature, skin lesions were not reported.

Thrombotic microangiopathies in humans

TMA is a term used to describe a pathological process which includes endothelial damage and thrombosis within the microvasculature, with high stress leading to platelet aggregation, a consumptive thrombocytopenia and red cell shearing, resulting in a microangiopathic hemolytic anaemia.[7] These features lead to ischemia and infarction which particularly affect the kidney. TMA is a histopathological diagnosis and features of this are identified in a number of specific clinical conditions including HUS, atypical hemolytic uremic syndrome (a-HUS) and thrombotic thrombocytopenic purpura (TTP).[7] TMA may be associated with underlying conditions such as malignancy, administration of chemotherapy and other drugs, transplantation, sepsis and disseminated intravascular coagulation.[8,9]

Hemolytic uremic syndrome

HUS in humans is triggered by infectious organisms and/or their toxins, for example shiga toxin-producing bacteria (e.g. *Escherichia coli* (STEC) and *Shigella dysenteriae* Type 1) or infection with *Streptococcus pneumoniae* (pneumococcal or p-HUS). However, in some individuals HUS is not infection- or toxin-triggered, and is therefore referred to as 'atypical' (a-HUS). In human patients with STEC-HUS, renal failure is commonly associated with prodromal hemorrhagic diarrhea and is most commonly recognized in children.[8] In this condition, shiga toxin binds with high affinity to globotriaosylceramide 3 receptors expressed on glomerular endothelial cells.[10] Injury to the glomerular endothelium precipitates a prothrombotic cascade leading to microthrombi formation.[7] Diagnostic investigations in these patients typically includes faecal culture and evaluation for *E.coli* endotoxin antibodies (IgM).[11]

Non-infection associated triggers, including genetic or acquired risk factors for defective regulation of the alternative complement pathway (AP), play a role in the pathogenesis of aHUS. Clinical features of a-HUS can be indistinguishable from other causes of TMA, with renal involvement predominating. Prodromal diarrhea is reported in up to 25% of patients ultimately considered to have aHUS.[12,13] AP complement dysregulation involves uncontrolled complement activation as a result of deficient or functionally impaired regulatory proteins or hyperactive C3 convertase components. It is hypothesized that the glomerular endothelium may be particularly susceptible to complement dysregulation, although the exact mechanism by which microthrombi formation ensues is not fully elucidated.[13] Identification of familial associations lead to the recognition of genetic predispositions which are identified in approximately 50-60% of individuals with aHUS.[9] These genetic variants have been identified in complement regulators (Factor H, Factor I, CD46 and

thrombomodulin) and complement activators (C3 and Factor B). Acquired autoantibody production against Factor H may also give a similar a-HUS phenotype, both with and without predisposing genetic variability in Factor H. Low plasma concentrations of C3 are consistent with a diagnosis of a-HUS but are neither specific nor sensitive, given the most common variants in complement factor H result in normal circulating C3 and Factor H concentrations.[7] Diagnosis of a-HUS in humans is therefore usually based on a combination of immunological and genetic testing. When a human patient presents with clinical signs compatible with a-HUS, initial investigations will include assessment of complement concentrations (C3, C4, Factor H and Factor I), mutation screening (*CFH, CF1, CD46, C3, CFB, THBD and DGKE*) and detection of factor H autoantibodies.[11]

Thrombotic thrombocytopenic purpura

TTP is an uncommon TMA which has been most commonly associated with acquired deficiency or genetic mutations in ADAMTS13.[7] ADAMTS13 is the cleaving protein of von Willebrands factor (vWF). vWF is required for primary hemostasis, facilitating platelet aggregation and thrombus formation at sites of endothelial injury. In health, vWF is gradually degraded into progressively smaller circulating forms by ADAMTS13 with the influence of shear stress. Since the 1980's it has been known that there is an association between TTP and highly thrombogenic 'ultra large' multimers of vWF, which was only later recognized to be due to deficiency or lack of functionality of ADAMTS13. Although genetic mutations in ADAMTS13 have been reported, these account for the minority of TTP cases and it is more common for ADAMTS13 deficiency to be the result of an acquired inhibitory autoantibody.[7] Very low ADAMTS13 activities are required for TTP, typically <5-10% of normal protease activity. However, in individuals where TTP is present in conjunction with co-existing TMA risk factors e.g. sepsis, malignancy, transplantation, then ADAMTS13 activity may not be markedly suppressed, so that quantification of ADAMTS13 is not a perfect diagnostic test.[11]

Interestingly, all three conditions have variable penetrance. For example, it is reported that some patients with hereditary forms of TTP do not manifest the disease until >20 years of age, not all patients infected with STEC will develop STEC-HUS, and only 40-50% of carriers of the recognized CFH, membrane cofactor protein and CFI mutations will manifest disease.[9] The pathogenesis of TMA is therefore complex and multifactorial, with interplay between genetic predispositions, environmental factors and other disease conditions determining whether an individual clinically manifests a disease phenotype. In humans, extensive exploration of clinical history, combined with advanced immunological and genetic diagnostic testing, is usually required in order to differentiate between causes of TMA.

Clinical presentation of UK cases of CRGV

Many different breeds of dog (>25, data courtesy of Anderson Moores Veterinary Specialists Ltd.) have been identified with CRGV, which is in contrast to American data, where the disease appeared to affect only racing greyhounds.[1,14,15]

Dogs diagnosed with CRGV are initially presented to veterinarians for assessment of skin lesions, affecting the limbs (77%), body (20%), face/muzzle (7%) and tongue (4%). Systemic signs are also sometimes present (Table 1), but rarely noted before the development of skin lesions (1% of cases; n=102, data courtesy of Anderson Moores Veterinary Specialists Ltd.).

Skin lesion appearance is variable, ranging from small, superficial abrasions (0.5cm), to large areas of full-thickness ulceration and necrosis (>30cm), with surrounding bruising and oedema. Lesions are commonly circular and erythematous. Central necrosis and ulceration develop subsequently. Lesions affecting digits frequently appear similar to pododermatitis or paronychia. Oral cavity lesions generally affect the tongue as circular erosions/ulcers, while lesions affecting the muzzle can be erosive/ulcerative or vesicular in appearance. Facial lesions commonly appear similar to acute moist dermatitis (Figure 1).

CRGV may cause skin lesions without systemic illness,[2] while some dogs develop AKI that may or may not lead to azotemia.[16] Currently, in the UK, the relative proportion of azotemic versus non-azotemic CRGV remains unknown, due to the voluntary nature of case reporting and the difficulties in achieving a definitive ante-mortem diagnosis. One case series (n=160 dogs) reported that 74% were non-azotemic[1]; however, diagnosis in these canine patients relied solely on clinical signs and expert opinion. In a further cases series (n=12), renal histopathology was performed in both azotemic and non-azotemic cases, via light and electron microscopy, and confirmed the presence of glomerular thrombotic microangiopathy in all dogs. The changes observed in the non-azotemic group were less severe and widespread than in the azotemic group.[14] In another report (n=18), 56% developed azotemia, while 44% remained non-azotemic, but diagnosis for all cases was reached by expert opinion based on clinical signs, blood and urine test results, without dermal or renal histopathology.[16]

Classification of CRGV into either non-azotemic or azotemic groups may be overly simplistic. The largest case series from the USA (n=160)[1] reported four possible clinical manifestations:

1) skin lesions with no systemic signs, remaining non-azotemic (a population potentially seen in the UK)

2) pyrexia and skin lesions, followed rapidly by azotemic AKI (this presentation appears to account for a low number of confirmed UK cases [19% of 102 cases[a]]
3) skin lesions with development of azotemic AKI within 10 days (60% of 102 cases[a])
4) azotemia prior to development of skin lesions (which appears rare in the UK; 1% of 102 cases[a])

In the UK, in 3% of dogs, azotemic AKI was detected more than 10 days post development of skin lesions (11, 20 and 21 days later[a]). This could suggest that another, delayed, clinical manifestation of azotemic CRGV exists, however, it is also possible that these cases developed AKI prior to biochemistry and urinalysis being performed.

<u>Approach to suspected cases and clinicopathological findings:</u>

Ante-mortem diagnosis of CRGV can be challenging. There is no single, non-invasive diagnostic test available with high sensitivity and specificity.

- **Renal histopathology:** Demonstration of TMA on renal histopathology is considered the reference standard for diagnosis. Fibrinoid necrosis and thrombosis, affecting the glomerular arterioles, is the most commonly reported finding with congestion of the glomerular tufts, and tubular degeneration and necrosis also reported.[4,1,14] Unfortunately, given the relative contra-indication for renal biopsy in dogs at risk of, or diagnosed with AKI and sometimes thrombocytopenia, this is usually obtained at post mortem with retrospective confirmation of CRGV.

- When presented with a dog for evaluation of skin lesions, which could be compatible with CRGV, baseline complete blood cell count, biochemistry and urinalysis, should be performed to assess for abnormalities which may further suggest CRGV. This also provides a baseline for ongoing monitoring. The frequency and duration of monitoring for development of AKI will depend upon many factors, including the level of clinical concern, owner preferences, dog temperament, and any financial considerations.

    - **Complete blood cell count:** This is normally unremarkable in dogs suspected to have non-azotemic CRGV, whereas the majority of cases that develop AKI (95%) have some combination of neutrophilia, non- or pre-regenerative anaemia and / or thrombocytopenia (Table 2). In these cases, blood smear examination may identify

[a] *Unpublished data, courtesy of Anderson Moores Veterinary Specialists Ltd.*

evidence of microangiopathic hemolysis (Burr cells, acanthocytes and / or schistocytes: 38% of 13 cases, Holm et al, 2015; 29% of 7 cases, Carpenter et al, 1988; 78% of 9 cases, Cowan et al, 1997).[1,4,16]

- **Biochemistry:** Serum biochemical analysis is generally unremarkable in suspected non-azotemic CRGV cases; however, mildly elevated serum liver enzyme activity has been identified (51/102 cases, median ALT activity 50u/L – range 35-117; reference range <25u/L).[17] Of the dogs developing AKI, 87-96% have abnormal serum urea and / or creatinine concentrations at the time of initial biochemistry. Azotemia has most frequently been documented 3 days after the development of skin lesions (n=102, range: 3 days prior to 21 days later). Other biochemical abnormalities include hyperphosphatemia, hyperbilirubinemia, elevated serum liver enzyme activity, mildly elevated serum muscle enzyme activity and mild hypoalbuminemia (Table 3). Abnormal canine pancreatic lipase results have also been identified (79% of 14 cases, unpublished data, courtesy of Anderson Moores Veterinary Specialists Ltd.).

- **IRIS AKI grading:** IRIS grading can be helpful to document severity of AKI but has not yet been shown to be prognostically significant for CRGV cases. Median IRIS AKI grade for UK CRGV cases (n=102) was III, range I-V.[17]

- **Urinalysis**: This is generally unremarkable in non-azotemic cases, although mild proteinuria has been reported (2 of 6 non-azotemic cases had UP: Cr > 1.0, Cowan et al, 1997; 14% of 13 cases had elevated UP: Cr, median value 0.85, range 0.56-1.14, reference range <0.5, Holm and Walker, 2018).[16,17] Commonly identified abnormalities in azotemic cases are similar to those seen in dogs with AKI of any cause, and include proteinuria (n = 5, UP:Cr 1.19-7.0, Cowan et al, 1997; n=102, median UP:Cr 3.42, range 1.81-7.64, reference <0.5), hematuria/hemoglobinuria (95%), glycosuria (32%) and granular or hyaline casts (n = 102).[17]

- **Oligoanuria:** Reduced or absent urine production is common in dogs with CRGV that developed AKI (urine output data available for 61 of 102 cases, revealed 70% were olig- or anuric, (30/61 oliguric and 13/61 anuric; unpublished data courtesy of Anderson Moores Veterinary Specialists Ltd.); 8 of 10 azotemic cases were olig- or anuric, Cowan et al, 1997). The authors are aware of 9 cases suspected to have had

CRGV, which developed severe AKI (median IRIS grade III; range II-V), but recovered with intensive management. Urine output data was available for 7 of these cases: 3 had normal urine output, 4 were oliguric and none were anuric (unpublished data courtesy of Anderson Moores Veterinary Specialists Ltd.).

- **Abdominal ultrasonography:** is useful in azotemic dogs to further assess renal architecture and exclude other post-renal causes of azotemia. Findings tend to be largely unremarkable in CRGV cases. Hyperechoic renal cortices are sometimes identified (~1/3 of cases, unpublished data courtesy of Anderson Moores Veterinary Specialists Ltd.) and some dogs have small volume abdominal effusions (due to hemorrhage, or volume overloading).

- **Dermal histopathology:** Skin biopsy, with dermal histopathology, *may* help to confirm the diagnosis, and can be considered for suspected cases. However, it commonly reveals non-specific, ischemic changes, including ulceration of the epidermis with coagulative necrosis of the subjacent dermis. In just over 1/3 of cases with dermal histopathology performed as part of a post mortem examination, (39% of 89 cases, unpublished data, courtesy of Anderson Moores Veterinary Specialists Ltd.), fibrinoid necrosis and thrombosis was identified in the small dermal arterioles, demonstrating a TMA process, supporting the diagnosis of CRGV.

Etiopathogenesis explored to date

At this time, the cause of CRGV remains unknown. When CRGV was first recognised in greyhounds in the 1980's and 1990's, it was postulated that it was associated with the ingestion of uncooked ground beef,[16] and STEC strains have been isolated more frequently from the faeces of greyhounds with CRGV than from healthy greyhounds. However, STEC strains were not isolated from all greyhounds diagnosed with CRGV.[18]

In the largest case series of dogs with CRGV to date, faecal culture was performed in seven dogs and yielded *E. coli*.[4] However, multiplex PCRs for *E. coli* virulence genes (eaeA, stx 1 and 2, LT1 and ST1 and 2) were negative in all of these dogs. Although shiga toxin has been identified in various species,[3,19] it has not been identified in dogs with HUS[5,20] and both fluorescent in situ hybridization (FISH; n=6) and PCR (n=4) for shiga toxin on renal tissue in the UK CRGV dogs were negative.[4] Reasons for failing to identify toxin, or causative bacteria, may have included previous antibiotic

238 administration, inappropriate sample handling, or late collection of samples. In humans, recovery of
239 toxin producing *E. coli* is highly dependent upon faecal culture being performed within six days of
240 the onset of diarrhoea.[21]
241
242 Other gram-negative bacteria including *Rickettsia rickettsii and* the leptospirae were postulated as
243 causative agents in Greyhounds; however, serology did not yield a definitive diagnosis.[1]
244 Leptospirosis was considered possible and has been explored further, although renal histopathology
245 in dogs with leptospirosis would not be compatible with CRGV.[22] Leptospirosis microscopic
246 agglutination testing (MAT) was performed in 15 cases.[4] Ten had negative titres, obtained a median
247 of three days (1–8 days) after the development of systemic signs but without available convalescent
248 titres. Five dogs had positive titres, albeit at a low concentration (1:100-1:800), and all of these dogs
249 had been vaccinated less than 1 year before testing. Although vaccinal titres often decline by four
250 months post-vaccination, they can sometimes persist for longer leading to false-positive results.[23]
251 Additionally, only single titres above 1:1600 are considered significant for indicating infection in
252 vaccinated dogs.[24] FISH and *Leptospira* PCR has been performed in low numbers of dogs confirmed
253 to have CRGV, but has yielded discordant results.[25]
254
255 In the UK case series, viral metagenomics was performed on fresh kidney tissue (n=2), liver (n=1),
256 and lymph node (n=1), by random nucleic acid amplification. All results were negative and
257 histopathologically there was no evidence of viral cytopathic effect (cytoplasmic inclusion bodies) in
258 any of the tissues examined.[4] PCR for canine circovirus was also performed on splenic tissue (n=4)
259 and blood (n=3) and FISH was performed on renal tissue (n=6); all results were negative.[26] Negative
260 results for viral metagenomics do not completely exclude a viral etiology. These results could
261 indicate that virus was present in low copy numbers, or that the virus was too remotely related to
262 known viruses used for sequence alignment, or that the sample used was too autolysed to preserve
263 the virus.
264
265 Renal tissue from two dogs was submitted to two separate laboratories, with both laboratories
266 receiving identical samples for evaluation with a broad spectrum set of 16S rRNA-directed probes.
267 One laboratory identified a clear 16S band in the tissue of one dog and a faint band in the other and
268 Staphylococcaceae were identified in both samples; however, this was thought to be the result of
269 contamination with commensal skin bacteria. Urine and renal tissue culture results were negative in
270 both dogs.[4] The second laboratory only identified leptospires in both samples.
271

A number of other aetiologies were considered in the UK case series; *Borrelia* PCR (n=5) and serology (n=2) were negative, and renal heavy metal concentrations (n=3; lead, arsenic and cadmium) were below reported reference intervals in all three.[4] A botanist from the Natural History Museum, London visited one of the sites that an affected dog had walked over the weeks prior to developing disease and no plant species observed were considered likely to be either causal or a co-factor in the development of CRGV (DW, personal communication). The only fungi identified have a long history in the UK and were considered unlikely to be the cause of this emerging disease. Urine toxicology was negative in five of six dogs tested. Pentaethylene glycol (trace) was detected in one dog.[4] A brown recluse spider bite was considered as a possible cause with a bite eliciting a pattern of necrotising dermatitis with subsequent vasculitis and necrosis in the kidney, but this spider is not endemic in the UK and arachnid envenomation would not correlate with the seasonality of the disease in the UK.[27,28] No evidence of ricinine was found in the urine of seven histopathologically confirmed cases (unpublished data courtesy of Anderson Moores Veterinary Specialists Ltd.).

Epidemiology of CRGV

The first known cases of CRGV in UK dogs were reported in 2012 and although initial numbers were very low (n=3), the annual frequency of reported cases showed a steady increase, albeit exhibiting occasional year-on-year variation. The outbreak pattern of CRGV in the UK is in accord with the definition of a newly emerging disease.[29] However, that does not mean that the disease was completely unknown in the UK, as it may simply not have been recognized, owing to a very low incidence in the population prior to 2012.

Breed risk factors

Previous non-UK studies have suggested that CRGV is associated primarily with greyhounds[1,2,14,16], with a single case reported in a Great Dane in Germany.[3] However, a comparison of 101 dogs diagnosed with CRGV between November 2012 and May 2017, with a denominator population of 446 453 UK dogs from the VetCompass™ database (VETCOMPASS 2014),[15] reported that greyhounds did not have a significantly higher odds of CRGV diagnosis (OR 1.65, $p = 0.629$) and that the disease was instead associated with multiple breeds. In general, hounds (OR 10.68, $p < 0.001$), gundogs (OR 9.69, $p < 0.001$) and pastoral dogs (OR 3.50, $p = 0.046$) had the highest risk of being diagnosed with CRGV, while toy dogs were absent from the case population. Compared with crossbreds, specific breeds with increased odds of being a CRGV case included the Flat-Coated Retriever (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) (Stevens and others 2018b).

306 Breeds with decreased odds of being a case were the Staffordshire Bull Terrier (OR 0.50), German
307 Shepherd Dog (OR 0.45) and Jack Russell Terrier (OR 0.37). Females were more likely to be
308 diagnosed with CRGV (OR 1.51), as were neutered dogs (OR 3.36).[15]
309
310 *Spatio-temporal distribution in the UK*
311 CRGV in the UK has been characterized by annual outbreaks which display a distinct seasonal
312 pattern. More than 90% of cases between 2012 and 2017 occurred between November and May,[30]
313 and Kuldorff's seasonal scan statistic identified a significant temporal cluster from December to April
314 (p=0.001; unpublished work). In general, negligible numbers of cases are reported during the
315 summer months.[30]
316
317 The number of cases has increased incrementally from 3 in 2012 to >60 in the 2017/2018 'season'.
318 The New Forest region on England's southern coast was the initial focus of the disease, although
319 cases have subsequently been identified across most of southern and western England.[30] The
320 eastern half of England however, has remained relatively free of the disease and is consequently
321 predicted to be a low-risk region.[30] Small, localised spatio-temporal clusters exhibiting significantly
322 higher proportions of cases than the rest of the UK were identified between February and March
323 2013 in the New Forest area (p = 0.004) and between January and April 2014 in Manchester (p =
324 0.087), although these clusters appeared to be transient, as they were not apparent every year.[30] In
325 fact, no cases were reported from the New Forest area in either 2016 or the 2017/2018 'season'.
326 Interestingly, between April 2015 and May 2017, the area immediately to the east of the New Forest
327 reported a significantly lower proportion of CRGV cases (p = 0.002) than the rest of the UK.[30]
328
329 *Agro-ecological risk factors*
330 A boosted regression tree model[30] identified habitat, specifically woodlands and lowland dry heath
331 communities, as the variable with the highest relative contribution to CRGV occurrence (20.3 %).
332 However, UK woodlands are highly diverse, each characterized by different types of trees, and
333 largely influenced by geology, soils, climate and history, making it difficult to identify a potential
334 source of the disease. Pastures were the habitat least associated with CRGV occurrence, suggesting
335 it is unlikely CRGV is the result of a livestock-related pathogen to which dogs are exposed while
336 walking across pastures. In addition to associations with specific habitat types, increasing relative
337 probability of CRGV presence was associated with increasing mean maximum temperatures in
338 winter, spring and autumn, increasing mean rainfall in winter and spring, and increasing mean
339 temperature in spring.[30] Stevens and others[30] suggest that appropriate climatic conditions on their

own appear to be insufficient for CRGV occurrence; the concomitant presence of suitable habitats appears to be essential, citing the fact that Wales and most of south-west England, where the disease has yet to gain any noticeable foothold, are dominated by pastures.

Management of CRGV cases

On the basis of our current understanding of CRGV, management is as for many dogs with AKI. Treatment goals are aimed at limiting further renal damage and enhancing cellular recovery.[31] Correction of fluid, electrolyte and acid-base disorders, achieving and maintaining normotension, and establishing/maintaining urine flow are the most important aspects of therapy.[32,33] Readers are directed to review articles for more specific information on the optimal medical management of AKI. Hypertension is a common complication of AKI.[34] The median blood pressure in dogs with CRGV was 176mmHg (range 102-280mmHg) at the time of onset of AKI.[4] Treatment is indicated if systolic blood pressure is >180mmHg or if there is evidence of 'end-organ' damage.[32,35]

<u>Immunomodulatory and anti-platelet therapy</u>

Management of human TMA's is dependent upon the underlying cause. Plasma therapy, antibiotic administration, monoclonal shiga toxin antibodies and renal transplantation have all been used in STEC-HUS. A recombinant, anti-C5 antibody (eculizumab) is the treatment of choice for human aHUS.[36,37,38] One dog with CRGV was reportedly ineffectively managed with immunosuppressive therapy.[3] The efficacy of monoclonal antibody therapy has yet to be evaluated in CRGV.

Anti-platelet therapy seems like a potential therapeutic consideration, given the etiopathogenesis of CRGV. Aspirin was part of the standard treatment protocol in the two largest studies of plasma exchange in TTP[39,40] and there have been reports of sudden deterioration and death among patients with TTP during recovery, when not taking platelet inhibitors.[41] Antiplatelet agents are usually not recommended for patients with TTP when bleeding is observed or when they also have severe thrombocytopenia. Low-dose aspirin is recommended by the British Committee for Standards in Hematology for patients with TTP with platelet counts greater than 50,000 per cubic millimeter.[42] Clopidogrel has been associated with the development of TTP.[43] Therefore, although there is no reported association with TTP in dogs, aspirin therapy may be preferred to clopidogrel.

<u>Wound management</u>

Skin lesions in CRGV should be appropriately managed once the dog is clinically stable; sedation or anaesthesia should be avoided for wound management unless deemed absolutely necessary, but

analgesia should be provided, taking into account potentially compromised renal function.[44,45,46,47] Debridement is rarely needed for lesions that develop in CRGV.[48,49] Samples for cytology and bacteriology should be collected, ideally before topical or systemic antimicrobial therapy is initiated.[50] Even if microorganisms are isolated from a lesion, the initiation of systemic antimicrobial treatment is contraindicated if there are no clinical signs that indicate infection.[51,52,53] If antimicrobial use is deemed appropriate, drug selection should initially be based on the most likely pathogen and their prevailing susceptibility patterns. Once the results of bacterial culture and sensitivity testing are available, the antimicrobial should be switched to the narrowest spectrum possible.[51] A sterile dressing should be applied to provide a physical barrier to prevent contamination and infection and to maintain a wound environment that accelerates wound healing. [50,54]

Utility of advanced therapies for CRGV cases

In many subtypes of TMA in people, the severity of the disease and underlying cause are not easily treatable.[55] Renal replacement therapy (RRT) offers ongoing support to reduce azotemia and maintain an appropriate fluid status and acid-base and electrolyte imbalance in patients with severely reduced kidney function, while awaiting either resolution of the AKI or renal transplantation.[55]

RRT has been used in dogs with CRGV with severe AKI resulting in oligo-anuria, and has allowed for treatment to be extended for a few weeks. However, this therapy alone, in the absence of specific treatments, has not been shown to be effective in improving survival in cases with severe renal damage (personal communication S. Cortellini). To date, there are no reports of the use of RRT beyond a few weeks in dogs; it is currently unknown whether providing RRT over a longer period may allow full recovery of the renal lesions induced by CRGV.

Given the high mortality associated with certain types of TMA in people, research efforts have been focused on mediating the dysregulation of the immune-system associated with these conditions. Implementation of alternative treatments has been necessary to target the activation of the complement system occurring in aHUS or the presence of anti-ADAMTS13 autoantibodies in TTP, as previously described in this review.

A novel immune-modulator, Eculizumab, a monoclonal antibody which binds to C5, impeding its hydrolysis and the subsequent activation of the complement pathway, has proven successful in treating aHUS compared to traditional immunosuppressive treatment.[55] This therapy, which is

currently highly recommended in people with aHUS, is cost-prohibitive for veterinary patients and there is no evidence for its efficacy in dogs with CRGV.

In people with anti-ADAMTS13 autoantibodies, therapeutic plasma exchange (TPE, also known as plasmapheresis) has been successful in improving survival rates.[55] This therapy consists of diverting blood in an extracorporeal circuit and removing plasma, either by filtration or by centrifugation of the blood, then replacing it with allogenic plasma from healthy donors. This therapy removes auto-antibodies, replaces ADAMTS-13, and reduces further activation of the coagulation system, thus limiting the progression of clinical signs. Current human guidelines advise the exchange of 1.5 times the plasma volume for each cycle, repeated every day until platelet numbers normalize.[56] Because this therapy has been proven effective in people with TTP, leading to a substantial increase in survival if performed in an early phase of the disease,[55] a recent case series study described the use of TPE in 6 dogs with severe CRGV.[57] While 2 dogs with severe AKI in the report survived, it still remains unknown if this therapy is superior to conservative management because of the uncontrolled study design. In addition, this therapy is usually effective in people with TTP if performed early and for extended periods, but dogs described in this study were already in an advanced stage of the disease and only received one or two treatments in total; hence, it remains unclear if this therapy could be useful if applied earlier in the disease.

Outcome and prognosis

Previous reports suggest that the prognosis for dogs with non-azotemic CRGV is excellent.[1,17] In the USA, dogs presenting with lethargy, pyrexia and skin lesions, with rapid development of AKI, also appeared to have a fair prognosis with intensive management, (25/30 survived). Dogs that developed azotemia before skin lesions were also reported to recover fully, (7/7 survived).[1] This is in contrast to the 100% mortality rate observed when dogs developed AKI within 10 days of the appearance of skin lesions,[1] which is similar to the experience in the UK, and also to later experiences in the USA, where the majority of dogs that developed significant azotemia were euthanized (100% of azotemic cases, Cowan et al, 1997; 83% of azotemic cases, Holm et al, 2015; 92% of azotemic cases, Holm and Walker, 2018).[4,16,17]

For UK cases, the median time from the development of skin lesions to euthanasia was 5 days (n =102; range 1-31 days, unpublished data, courtesy of Anderson Moores Veterinary Specialists Ltd.). Reasons for euthanasia included oligoanuria refractory to medical management (n=29), progressive azotemia (n=25), perceived poor prognosis (n=15), development of seizures (n=4), development of suspected

ALI / ARDS (n= 4), financial constraints (n=4), progressive anaemia (n=2), suspected DIC (n=2) and suspected sepsis (n=2). A further two dogs died and the reason for euthanasia was not stated for 13 cases. It is possible that prognosis for some cases could have been more favourable if more intensive management had been pursued, but this is unknown.

The authors are aware of a small number of suspected cases (n=3) that developed IRIS grade I AKI*, which responded well to intravenous fluid therapy +/- furosemide, and recovered uneventfully.[b] Again, the difficulty of confirming the diagnosis without renal histopathology has hampered efforts to better understand the true prognosis for CRGV in dogs in the UK. The small number of suspected cases with severe azotemia that survived could suggest that CRGV with AKI is not invariably fatal with appropriate intensive management.

Conclusions

CRGV is an emerging disease in the UK but the etiology and any association with an infectious agent remains uncertain at this time. A population of non-azotemic dogs with CRGV exists and for such cases, prognosis may be good. For the population of dogs that develop azotemia, and particularly oligoanuric AKI, the prognosis can be guarded, but intensive medical therapy is indicated in these cases as successful outcomes have been achieved. At this stage, further work must focus on the underlying infectious triggers and immune dysregulation which have been associated with similar TMA conditions in humans, in order to determine whether risk factors can be identified for the canine population.

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[b] *These cases either developed an increase in serum creatinine concentration (>26.4umol/L above baseline, while remaining within the reference range), or oliguria (UOP <1ml/kg/hr).*

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626 **Table 1. Clinical signs at initial presentation**

Clinical signs at initial presentation	Percentage of dogs affected (n=102)
Skin lesions	99
lameness	33
anorexia	28
vomiting	21
lethargy	18
pyrexia	17
Signs of bleeding	4
diarrhoea	3
Neurological signs	2
jaundice	1
hypothermia	1

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629 **Table 2. Selected hematology results for CRGV cases**

Publication	Abnormality	Number of dogs in report.	% of dogs affected	Median value	range	Normal reference range
Carpenter, et al 1988 (USA)	thrombo-cytopenia	7	86	103 x 10 9/L	45-241 x 10 9/L	-
	anaemia	7	71	41%	37-51%	>55%
Hertzke et al, 1995 (USA)	thrombo-cytopenia	12	100	43 x 10 9/L	<10-173 x 10 9/L	>200 x 10 9/L
Cowan et al, 1997 (USA)	thrombo-cytopenia	18; 10 azotemic, 8 non-azotemic	100	Mean values: azotemic 43.9 x 10 9/L Non-azotemic 114.6 x 10 9/L	Azotemic cases: 6-97 x 10 9/L Non-azotemic: 6->120 x 10 9/L	>180 x 10 9/L
	anaemia	18; 10 azotemic, 8 non-azotemic	100% of azotemic cases 75% of non-azotemic cases	Mean values: azotemic 29% Non-azotemic 47%	-	-
	neutrophilia	18; 10 azotemic, 8 non-azotemic	-	Mean values: azotemic 17.053 x 10 9/L Non-azotemic 8.881 x 10 9/L 47%	-	-
Holm et al, 2015 (UK)	thrombo-cytopenia	30	50	78 x 10 9/L	1-401 x 10 9/L	175-500 x 10 9/L
	anaemia	30	23	43.9%	26-65.3%	37-55%
Holm and Walker, 2018 (UK)	thrombo-cytopenia	102.	78% (n=76 cases)	40 x 10 9/L	0-60 x 10 9/L	175-500 x 10 9/L
	anaemia	102.	22 (n= 22 cases)	30.6%	24-36%	37-55%
	neutrophilia	102.	52 (n= 53 cases)	13.7 x 10 9/L	10.6-37.9 x 10 9/L	2.8-10.5 x 10 9/L

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632 **Table 3. Selected biochemistry results for CRGV cases**

Publication	Abnormality	Number of dogs in report.	% of dogs affected	Median value	range	Normal reference range
Carpenter, et al 1988 (USA)	Elevated Urea / BUN	7 data available for 5	44 of 168 (26%)	105 mg/dl (37.5 mmol/L)	24-453 mg/dl (15-161.8 mmol/L)	-
	Elevated Creatinine	7 data available for 5	44 of 168 (26%)	7.2 mg/dl (636.5µmol/L)	2.2-23.3 mg/dl (194.5-2059.7 µmol/L)	-
	Increased ALT activity	7	5 of 7 (71%)	-	74-510 u/L	-
Hertzke et al, 1995 (USA)	Elevated Urea / BUN	12	7 of 12 (58%)	240 mg/dl (85.7 mmol/L)	80-450 mg/dl (28.6-160 mmol/L)	<40 mg/dl
	Elevated Creatinine	12	7 of 12 (58%)	5.6 mg/dl (495µmol/L)	2.5-19.6mg/dl (221-1732.7 µmol/L)	<2.0 mg/dl
Cowan et al, 1997 (USA)	Elevated Creatinine	18	10 of 18 (56%)	-	-	>1.8 mg/dl
	Increased ALT activity	18	11 of 18 (61%)	-	-	-
	Increased CK activity	18 data available for 15	3/8 non azotemic (38%) 6/7 azotemic (86%)	Mean in non-azotemic cases 556u/L Mean in azotemic cases 4136u/L	-	-
	Hypo-albuminemia	18	2/8 non-azotemic (25%) 9/10 azotemic (90%)	Mean in non-azotemic cases 2.33g/dl Mean in azotemic cases 1.66g/dl	-	-
Holm et al, 2015 (UK)	Elevated Urea / BUN	30	100%	46.4mmol/L	3.6-85.1 mmol/L	2.0-9.0 mmol/L
	Elevated Creatinine	30	100%	406.5µmol/L	71-900 µmol/L	40-159 µmol/L
	Increased ALT activity	30 data available for 25	21/25 (84%)	119 u/L	48-950 u/L	<100 u/L
	Increased ALKP activity	30 data available for 28	11/28 (39%)	91.5 u/L	16-650 u/L	<212 u/L

	Hyper-bilirubinemia	30 data available for 27	9/27 (33%)	12umol/L	0-338 µmol/L	0-15 µmol/L
	Hyper-phosphatemia	30 data available for 26	21/26 (81%)	3.12mmol/L	1.28-6.2 mmol/L	0.8-2.20 mmol/L
	Increased CK activity	30 data available for 8	4/8 (50%)	206u/L	112-881 u/L	<190 u/L
	Increased AST activity	30 data available for 6	6/6 (100%)	76.5u/L	51-473 u/L	<49 u/L
	Hypo-albuminemia	30 data available for 27	10/27 (37%)	27g/L	14-36 g/L	26-40 g/L
Holm and Walker, 2018 (UK); and some unpublished data, courtesy of Anderson Moores Veterinary Specialists Ltd.	Elevated Urea / BUN	102	98/102 (96%)	41.9 mmol/L (n = 102)	4.7-92.6 mmol/L (n = 102)	2.0-9.0 mmol/L
	Elevated Creatinine	102	96/102 (94%)	304 µmol/L (n = 102)	68-1606 µmol/L (n = 102)	40-106 µmol/L
	Increased ALT activity	102 data available for 83	79/83 (95%)	199 u/L (n = 79)	34-950 u/L (n = 79)	<25 u/L (n = 24) <100 u/L (n = 59)
	Increased ALKP activity	102 data available for 86	37/86 (43%)	335 u/L (n = 37)	76-2920 u/L (n = 37)	<50 u/L (n = 24) <212 u/L (n = 62)
	Hyper-bilirubinemia	102 data available for 76	51/76 (67%)	27 µmol/L (n = 51)	16-603 µmol/L (n = 51)	<15 µmol/L
	Hyper-phosphatemia	102 data available for 83	65/83 (78%)	3.42 mmol/L (n = 65)	1.81-7.64 mmol/L (n = 65)	0.8-1.6 mmol/L (n = 30) 0.81-2.2 mmol/L (n = 53)
	Increased CK activity	102 data available for 17	12/17 (71%)	295 u/L (n = 12)	217-881 u/L (n = 12)	<190 u/L
	Increased AST activity	102 data available for 10	10/10 (100%)	86 u/L (n = 10)	51-473 u/L (n = 10)	<49 u/L

	Hypo-albuminemia	102 data available for 85	24/85 (20%)	23 g/L (n = 24)	14-25 g/L (n = 24)	26-40 g/L

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635 **Figure legends**
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637 ***Figure 1: Typical skin lesions identified in dogs with CRGV***
638 *1a) Interdigital skin lesion identified in dog with CRGV*
639 *1b) Progressive ulcerated skin lesions identified in dog with CRGV*
640 *(Photographs courtesy of Dr Stefano Cortellini)*
641