Canine coronavirus subtype 2a associated with outbreaks of fatal diarrhoea in bush dog *(Speothos venaticus)* groups

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https://doi.org/10.17236/sat00320

Eingereicht: 24.11.2020
Angenommen: 10.05.2021

Fatale Diarrhoe bei Waldhunden *(Speothos venaticus)* durch Canines Coronavirus Subtyp 2a

In verschiedenen Gruppen von Waldhunden *(Speothos venaticus)* aus zwei zoologischen Einrichtungen traten zwischen 2009 und 2017 drei Ausbrüche von tödlicher Diarrhoe auf. Die auffälligsten klinischen Symptome waren Durchfall, Anorexie und reduzierter Allgemeinzustand. Trotz Therapie kam es bei jedem Ausbruch zu einer Reihe von Todesfällen. Die auffälligsten makroskopischen pathologischen Befunde waren Abmagerung, Erytheme, Blutungen in den Schleimhäuten, und Ulzerationen im Magen-Darm-Trakt. Zu den histopathologischen Befunden gehörten der Verlust und die Verschmelzung der Darmzotten, Verlust des Kryptenepithels und die lymphatische Depletion. Diese Befunde unterstützten eine virale Ätiologie und führten zur Verdachtsdiagnose einer Caninen Coronavirus Infektion. Die Diagnose wurde anhand der Serologie (steigende Antikörpertiter) und mittels Polymerase-Kettenreaktion (Nachweises viraler Nukleinsäure) bestätigt. Das Canine-Coronavirus wurde als Typ 2a subtypisiert, welches bei juvenilen Haushunden nachweislich zu einer systemischen tödlichen Erkrankung führen kann. Der vorliegende Fallbericht beschreibt nach bestem Wissen der Autoren die ersten Fälle von tödlichem Durchfall durch das Canine Coronavirus Typ 2a bei Waldhunden. Diese Ausbrüche deuten darauf hin, dass adulte Waldhunde sehr empfänglich für das Canine Coronavirus sind und einer viralen Enteritis erliegen können.

Schlüsselwörter: Canidae, Magen-Darm-Trakt, Enteritis, Coronavirus.

Summary

Three outbreaks of fatal diarrhoea occurred in bush dog *(Speothos venaticus)* groups at two zoological collections in the United Kingdom between 2009 and 2017. In all cases, the predominant clinical signs were diarrhoea, anorexia and severe loss of condition. Despite supportive treatment, a number of fatalities occurred during each outbreak. Common gross post mortem findings were emaciation, with erythema, mucosal haemorrhage, and ulceration of the gastrointestinal tract. Histopathological features included villus blunting and fusion, crypt epithelial loss and lymphoid depletion, supporting a viral aetiology and canine coronavirus was suspected. Diagnosis was confirmed on the basis of serology (rising antibody titres) and the detection of viral nucleic acid using polymerase chain reaction. The canine coronavirus was subtyped as type 2a, which is known to cause systemic fatal disease in immature domestic dogs. To the authors' knowledge, these are the first reported cases of fatal diarrhoea associated with canine coronavirus type 2a in bush dogs. These outbreaks suggest that adult bush dogs are highly susceptible to canine coronavirus infection and may succumb to viral enteritis.

Keywords: Canid, gastrointestinal tract, enteritis, coronavirus.

Introduction

The bush dog *(Speothos venaticus)* is a small canid native to Central and South America. It is found in multiple habitats but is generally associated with water sources. It is classified as near threatened on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species.[22]

The European captive population currently contains 176 individuals held at 40 institutions. There is very little published information on disease in wild and captive bush dogs. Diarrhoea has been anecdotally reported as a common occurrence in captive populations. Fifty percent of respondents to the 2012 Bush Dog Studbook Survey reported to have seen diarrhoea in their bush dog groups.[5] In the majority of cases, the cause remained undiagnosed.

A common and well documented cause of diarrhoea in the domestic canine population is canine coronavirus (CCoV).[4,34,46] With the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV2), coronaviruses have had an increased international interest. Coronaviruses are currently classified into three different antigenic groups, 1 to 3. CCoV is classified as a group 1 coronavirus, unlike the SARS-like coronaviruses which are group 2.[16] They are, therefore, not considered to be closely related. Group 3 coronaviruses are those of avian origin.[16] Similar to other coronaviruses, CCoV can mutate readily and a number of strains of canine coronavirus have been recognised. Type 1 and 2 are the predominant strains circulating currently. Clinical disease is usually mild and generally occurs in young canids.[44] Two type 2a CCoV biotypes currently exist, enteric and pantropic.[14] Infections with the enteric form in domestic dogs, are characterised by high morbidity and low mortality and are generally restricted to the gastrointestinal tract.[16] Pantropic CCoV was first identified in 2006; in domestic dogs, it is associated with fatal systemic infection and is more virulent than enteric forms.[6,17] Evidence of canine coronavirus has previously been reported in wild canids including grey wolves *(Canis lupus)*, and coyotes *(Canis latrans)* as well as in wild maned wolves *(Chrysocyon brachyurus)*, pampas foxes *(Pseudalopex gymnocerus)* and crab-eating foxes *(Cerdocyon thous)* in the bush dog's natural range.[11,25,29,35,47] It is likely that bush dogs have a similar disease susceptibility to other wild and domestic canids, however, data to support this is scarce.

This case study describes outbreaks of CCoV infection in three bush dog groups, during the period of 2009 to 2017. This suggests this virus is an important pathogen in the development of severe and fatal diarrhoea in captive bush dogs. To the authors' knowledge, these are the first recorded cases of disease associated with CCoV in the bush dog.

Table 1: Clinical details of individual outbreaks of Canine coronavirus in bush dogs *(Speothos venaticus)* including signalment, clinical signs, clinical pathology, therapies, deaths and recovery period.

Outbreak, date and institution	ID	Age/ sex	Clinical signs	Relevant history and clinical pathology	Individual clinical pathology	Therapies	Hospitalised	Further treatment	Survived/ died	Time to recovery/ death (days)
Outbreak 1 2009 Institution 1	1	4y10m Male	DAWI Vomiting	Distemper and parvovirus vaccinated Faecal parasitology and culture negative	Haematology and biochemistry performed (see table 2) Parvovirus antibody titre protective (640)	Amoxycillin-Clavulanic acid (20mg/kg i.m sid 7d) Enrofloxacin (5mg/kg i.m sid 7d) Ranitidine (2mg/kg i.v tid 5d) Dexamethasone (0.2mg/kg s.c sid 10d) Lactated ringers solution (40ml i.v sid 10d) Glucose (4ml/kg i.v sid 5d) Probiotics (p.o sid 14d)	N	–	Survived	104
	2	4y4m Female	DAWI Vomiting		–		N	–	Survived	104
	3	0y10m Female	DAWI Vomiting		Haematology and biochemistry performed (see table 2)		N	–	Survived	104
	4	0y10m Male	DAWI Vomiting		–		N	–	Survived	104
	5	0y10m Female	DAWI Vomiting Pale mucous membranes		Haematology and biochemistry performed (see table 2)		N	–	Died	30
	6	0y10m Male	DAWI Vomiting Collapse		Haematology and biochemistry performed (see table 2) High CCoV antibody titre at time of death (1280)		Y	Lactated ringers solution (100ml/kg/ day CRI)	Died	37

Outbreak, date and institution	ID	Age/ sex	Clinical signs	Relevant history and clinical pathology	Individual clinical pathology	Therapies	Hospitalised	Further treatment	Survived/ died	Time to recovery/ death (days)
Outbreak 2 2017 Institution 1	7	6y6m Male	DAWI Dehydration Depression Jaundice	Distemper and parvovirus vaccinated Faecal parasitology negative Faecal culture campylobacter positive Faecal canine parvovirus PCR negative Faecal CCoV PCR positive CCoV subtype 2a	–	Amoxycillin-clavulanic acid (15mg/kg i.m sid 7d) Ceftazidime (8mg/kg s.c once) Tylosin (2g/4l p.o in water sid; 10mg/kg i.m sid 5d) Probiotics (p.o sid 28d) Rehydration solution (p.o sid in water)	Y	Lactated ringers solution (100ml/kg/day constant i.v infusion) Omega interferon (2.5MU/kg i.m sid 3d)	Died	42
	8	4y9m Female	DAWI Dehydration Depression Collapse		–		Y	Lactated ringers solution (100ml/kg/day constant i.v infusion) Metronidazole (10mg/kg i.v bid 5d) Ranitidine (2mg/kg i.v tid 5d)	Died	15
	9	2y1m Male	DAWI		–		N	–	Survived	63
	10	2y1m Female	DAWI		–		N	–	Survived	63
	11	2y1m Female	DAWI		–		N	–	Survived	63
	12	1y1m Male	DAWI		–		N	–	Survived	63
	13	1y1m Male	DAWI		Haematology and biochemistry performed (see table 2) Parvovirus antibody titre protective (512) Rising coronavirus antibody titre over 10 days (0 to >1280)		N	–	Survived	63
	14	1y1m Male	DAWI Dehydration Depression		–		Y	Lactated ringers solution (100ml/kg/day constant i.v infusion) Metronidazole (10mg/kg i.v bid 5d) Ranitidine (2mg/kg i.v tid 5d) Omega interferon (2.5MU/kg i.m sid 3d)	Died	28
	15	1y1m Male	DAWI Dehydration Collapse		–		Y	Lactated ringers solution (100ml/kg/day constant i.v infusion) Metronidazole (10mg/kg i.v bid 5d) Ranitidine (2mg/kg i.v tid 5d)	Died	14
	16	1y1m Male	DAWI Dehydration Collapse		Haematology and biochemistry performed (see table 2)		Y	Lactated ringers solution (100ml/kg/day constant i.v infusion) Metronidazole (10mg/kg i.v bid 5d) Ranitidine (2mg/kg i.v tid 5d)	Died	17
	17	0y2m Female	DAWI		–		N	–	Died	63
Outbreak 3 2017 Institution 2	18	2y10m Female	DAWI	Faecal parasitology and culture negative Faecal canine parvovirus PCR negative Faecal CCoV PCR positive CCoV subtype 2a	–	Amoxycillin-clavulaic acid (12.5mg/kg p.o bid 7d) Metronidazole (25mg/kg p.o bid 5d) Potassium supplementation (2ml/4.5kg p.o bid 7d) Probiotics (p.o sid 14d)	Y	Lactated ringers solution (60ml/kg/day i.v CRI)	Died	Info required
	19	2y5m Male	DAWI		–		Y	Lactated ringers solution (60ml/kg/day i.v CRI)	Died	Info required

DAWI=diarrhoea, anorexia, weight loss

Case report

Three outbreaks of acute diarrhoea, anorexia and weight loss occurred in separate groups of bush dogs between 2009 and 2017. A full summary is provided in Table 1. The bush dogs were captive bred and housed at zoological institutions in the United Kingdom. The founding individuals of each group were unrelated and originated from other European institutions. They were housed in enclosures with both outdoor and indoor access and were fed on a diet of whole prey items. The groups had no contact with each other or other canids prior to and during the outbreak period. No new individuals were introduced to any group in the preceding twelve months. All individuals in all 3 groups were affected (n=19) across all three outbreaks. These were eight females and eleven males aged between two months and six years. Seventeen individuals were vaccinated against canine distemper and canine parvovirus annually. No individuals were vaccinated against CCoV.

Disease development and progression were consistent across all outbreaks. All cases presented with initial signs of acute diarrhoea and anorexia. The length of the outbreaks from initial clinical signs to complete resolution varied from 63 to 104 days and deaths occurred between 14 and 42 days. Vomiting was initially observed in individuals in outbreak 1 but resolved within 48 hours. Marked weight loss became apparent in all individuals as the disease progressed. Ten individuals were mildly affected and did not exhibit any further clinical signs. Nine individuals were severely affected and developed dehydration, lethargy and four cases collapsed.

Diagnostic testing was performed to identify the causative agent. Faecal testing was performed on pooled

Table 2: Blood haematology and biochemistry results of bush dogs *(Speothos venaticus)* sampled during canine coronavirus type 2a outbreaks.

	Individual					
	1	3	5	6	13	16
Status	**Survived**	**Survived**	**Died**	**Died**	**Survived**	**Died**
Haemoglobin (g/dl)	14.5	17.6	13.9	12.9	15.3	15.1
Haematocrit (l/l)	0.41	0.47	0.39	0.36	0.40	0.41
RBC (1012/l)	5.03	6.23	4.98	4.65	5.53	5.43
MCV (fl)	81.5	75.4	78.3	77.4	72.3	75.5
MCHC (g/dl)	35.4	37.4	35.6	35.8	38.3	36.8
MCH (pg)	28.8	28.3	27.9	27.7	27.7	27.8
WBC (109/l)	17.7	12.7	13.6	14.3	19.6	1.3
Segmented neutrophils (109/l)	15.58	10.41	11.42	13.16	15.88	0.91
Banded neutrophils (109/l)	0.18	0.00	0.00	0.00	0.00	0.00
Lymphocytes (109/l))	1.42	1.91	0.82	0.57	2.55	0.35
Eosinophils (109/l)	0.00	0.00	0.00	0.00	0.39	0.00
Monocytes (109/l)	0.53	0.38	1.36	0.57	0.78	0.04
Basophils (109/l)	0	0	0	0	0	0
Platelets (109/l)	193	258	439	439	278	100
Total protein (g/l)	79.9	81.6	62.4	67.4	75.0	72.0
Albumin (g/l)	41.0	46.2	39.2	38.6	33.0	33.0
Globulin (g/l)	38.9	35.4	23.2	28.8	42.0	39.0
AG ratio	1.05	1.31	1.69	1.34	0.80	0.80
Urea (mmol/l)	34.0	27.3	47.2	81.8	6.6	122.0
Creatinine (µmol/l)	110	115	105	163	35	541
ALT (µ/l)	67	49	146	96	89	158
ALP (µ/l)	28	71	53	35	18	79
Glucose (mmol/l)	8.64	7.10	8.37	7.20	6.60	20.60
Cholesterol (mmol/l)	4.35	4.91	1.96	2.41	3.90	4.30
Calcium (mmol/l)	2.51	2.86	2.87	2.65	2.43	–
Total bilirubin (mmol/l)	2.4	1.2	0.9	0.9	2.0	32.0
Amylase (µ/l)	171	126	190	168	149	289
Sodium (mmol/l)	134.6	131.9	136.4	131.7	136.0	124.0
Potassium (mmol/l)	4.97	5.36	4.00	4.28	4.20	3.50
Na:K ratio	27.1	24.6	34.1	30.8	32.0	35.0

samples in all three outbreaks. McMasters faecal floatation was negative. *Campylobacter* spp. were identified in individuals in outbreak 2, but not further speciated. No pathogenic bacteria were isolated from the other eight individuals in outbreak 1 and 3. Biochemistry and haematology was performed in six individuals, and blood value alterations were very similar in all three outbreaks (Table 2). Elevated urea was the most common biochemical finding with five of the six sampled individuals exhibiting this abnormality. A Kruskal-Wallis test was performed and did not identify any statistically significant differences for any blood parameters between the three individuals that survived and the three fatalities ($p > 0.05$).

Treatment was administered to all nineteen individuals. Mildly affected individuals (n = 10) received antibiotics, probiotics, and rehydration solution. Severely affected individuals (n = 9) which developed signs of dehydration, depression or collapse received antibiotics, fluid therapy, and ranitidine (see Table 1). All severely affected animals either died or were euthanised due to their deteriorating condition and poor prognosis. A total of nine individuals across the three outbreaks were fatally affected. The age range of fatalities was between one and six years.

A gross necropsy was performed on all fatalities (n = 9). Emaciation was evident in 6/9 individuals. In the majority of cases (6/9), gross changes including erythema, mucosal haemorrhage, and ulceration, were evident in either the stomach, small intestine, large intestine, or a combination of these (Figure 1). Minimal gross changes were evident in three individuals. The majority of histopathological lesions were within the gastrointestinal tract with gastritis, enteritis, colitis or a combination thereof evident in all individuals (Figure 2). These were typically characterised as lymphocytic or mixed cellular. Lymphoid depletion of the spleen and/or mesenteric lymph node was also a predominant feature, present in 5/9 cases (Figure 3). Additional findings were also present in fatalities from outbreak 2 included pneumonia (4/9) and necrotising hepatitis (3/9). Accompanying gross changes of affected organs were also seen in these cases.

Canine parvovirus and coronavirus testing was performed using polymerase chain reaction-based assays on fresh faecal samples representing each outbreak (total n=13). In order to extract viral RNA for genotyping, a 100 mg aliquot of faeces was first mixed in 1 mL of 10 M guanidine thiocyanate in Tris HCL containing 3% W/V polyvinylpolypyrrolidone (PVPP) to lyse the faecal matter (All chemicals listed Fisher Scientific, Loughborough, UK). The supernatant from the lysis step was loaded into a Taco™ Automated Nucleic Acid Extraction System (GeneReach, Taiwan) and the purified RNA eluted into 180 µl of elution buffer. Parvovirus PCR was negative in each case while quantitative reverse-transcriptase PCR (qRT-PCR) detected a high level of CCoV ribonucleic acid (RNA) in each sample. To further investigate the strain of canine coronavirus involved in outbreaks 2 and 3, the faeces used to perform the original diagnostic PCR which had been archived frozen was used for CCoV subtyping. No faeces was available from outbreak 1 to perform subtyping. CCV subtype 2a was confirmed as the canine coronavirus involved in outbreaks 2 and 3 (see Figure 4 for full methodology). Rising serum antibody titres for CCoV were present in the one individual tested, rising from 0 to >1280 over a two week period.

Discussion

This case series describes three isolated outbreaks of diarrhoea resulting in several fatalities in three bush dog groups housed at zoological institutions in the United Kingdom.

Diarrhoea was one of the predominant clinical sign in these cases. It is a common clinical sign in domestic dogs with multiple possible causes such as husbandry (including dietary) factors and pathogens.[28,43] A dietary cause for these outbreaks was investigated, but rapidly excluded. No dietary changes had occurred, the diet in all groups was consistent and unchanged over the

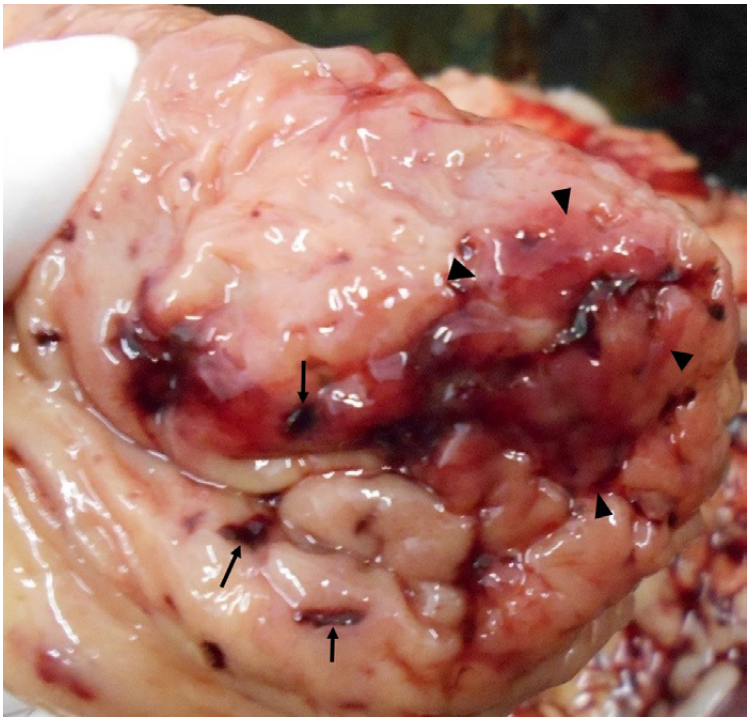

Figure 1: A gross necropsy image of the stomach mucosa of a bush dog *(Speothos venaticus)* which died following canine coronavirus type 2a infection. Note the mucosal haemorrhage (arrow heads) and ulcerations (arrow) present.

preceding twelve months. Bacterial and viral contamination of food was also excluded as food items were of high quality and stored and prepared hygienically. In addition, faecal bacteriology did not identify common bacterial pathogens except in outbreak 2.

In outbreak 2, *Campylobacter* spp. was identified on faecal culture. Campylobacter has been identified as a causal agent of diarrhoea in domestic dogs.[32,42] However, it has also commonly been isolated from the gastrointestinal tract of asymptomatic individuals.[7,8,27,45] The *Campylobacter* in this case was not speciated, therefore, it is not known if the strain present was considered pathogenic or non-pathogenic. However, it was ruled out as the cause of disease in these cases as the intestinal pathology was consistent with a viral aetiology.

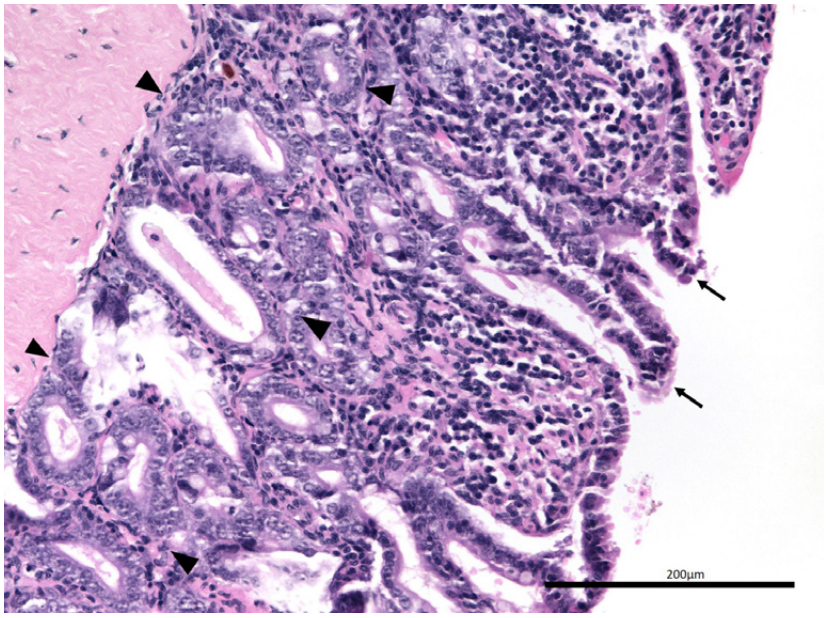

Figure 2: Lymphocytic enteritis with villus blunting (thin arrows), crypt degeneration and regeneration (area between arrow heads) in the small intestine in a bush dog (*Speothos venaticus)* which died during a canine coronavirus type 2a outbreak. Haematoxylin and eosin, original magnification x 200.

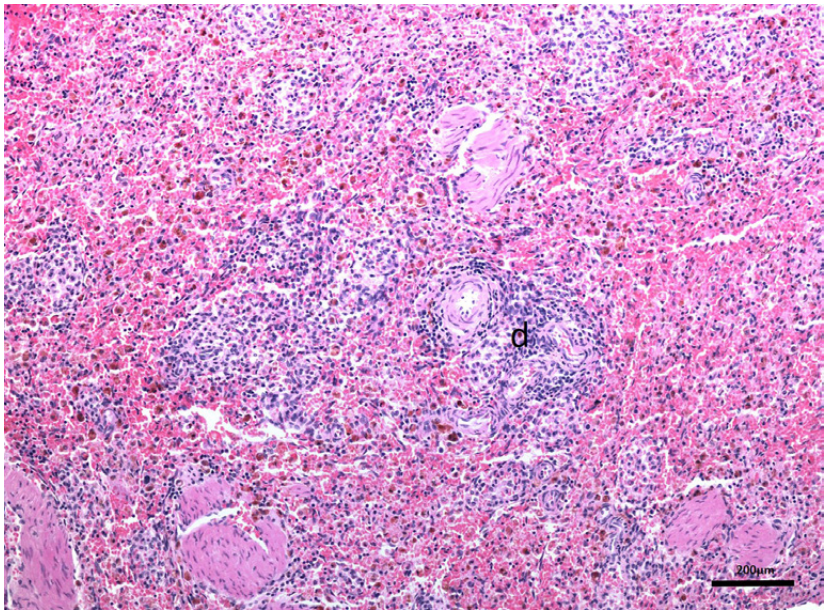

Figure 3: Spleen exhibiting marked periarteriolar lymphoid depletion (d) from a bush dog *(Speothos venaticus)* with canine coronavirus type 2a outbreak. Haematoxylin and eosin, original magnification x 100.

Common histopathological changes were seen in the enteric and lymphoid systems of fatalities from all outbreaks. In outbreak 2, pathology of other organs systems, including the lower respiratory tract and liver, were also present. In all cases, histopathological analysis identified the cause of the pathology in these other organ systems as a mixed gram negative bacterial infection. The authors' suspect this to be an opportunistic infection secondary to the pre-existing CCoV infection. It is likely *Campylobacter* spp. was one of the bacterial agents involved but no bacteriology was performed to confirm this.

Pneumonia was identified pathologically in fatalities from outbreak 2. Another coronavirus, Canine Respiratory Coronavirus (CRCoV) has been shown to be present in the UK and is associated with pathological respiratory tract changes.[23]. As CRCoV and enteric CCoV are genetically distinct, the PCR performed in these bush dog cases was specific to enteric CCoV, confirming enteric CCoV as the cause of disease. The respiratory pathology was also consistent with a secondary bacterial infection making a CRCoV infection in the respiratory tract unlikely.

The common enteric histopathological features in these outbreaks were intestinal villus blunting and fusion, crypt epithelial loss and ulcerative gastritis. These findings were indicative of a viral pathology but not pathognomic for canine coronavirus. Histological differential diagnoses include other enteric viral diseases such as parvovirus,[9] as well as both enteric[30] and pantropic strains of canine coronavirus.[33] The other prominent feature in this case series was lymphoid depletion of the spleen and mesenteric lymph nodes. This is a common histopathological finding in both parvovirus[38] and pantropic coronavirus infections.[48] Therefore, it is impossible to differentiate parvovirus from coronavirus on histopathology alone. It is recommended to perform both parvovirus and coronavirus PCR in cases with similar histopathological findings.

PCR confirmed the presence of CCoV in these cases and molecular subtyping identified it as type 2a. Fatalities can be seen with both enteric and pantropic biotypes. Fatal disease in those infected with the enteric biotype has only been reported as a result of mixed

infections with other viral pathogens, such as canine parvovirus.[13,15] In the present case series, concurrent parvovirus infection was excluded so a mixed infection seems unlikely. In the absence of any concurrent viral infections, the fatalities and pathology exhibited in these cases are consistent with a pantropic biotype. The two biotypes are genetically indistinct, therefore, the only way to definitively diagnose the presence of a pantropic biotype is detection of CCoV in internal organs by PCR.[20] The authors' suspect the presence of pantropic CCoV but unfortunately, tissue samples were not available to confirm this.

In reports from domestic dogs, only juvenile individuals suffered fatalities with mixed viral infections involving enteric CCoV,[15,39] and only individuals under six months of age were affected by pantropic CCoV, with fatal outcomes most commonly seen in the youngest animals.[17] In contrast, a wide range of age groups were affected in this case series, and the majority of fatalities were adults. The youngest individual affected was two months old and did not present as severely ill as the majority of adults. The reason for the adult mortalities in this case series remains subject to speculation. It is possible that more severe disease occurred due to viral exposure in a naïve population. However, CCoV type 2a has been shown to be circulating in domestic dogs throughout Europe and South America.[2,10,21,37] Based on this, complete naivety of both the captive and the wild bush dog population seems unlikely. Alternatively, the bush dog evolved independently of the domestic dog and diverged from the common canine ancestor approximately three million years ago.[36] Therefore, this could be the result of a genetically-restricted or host-species specific phenomenon. Further research would be required into this apparent increased susceptibility in this species.

The source of the CCoV infection is currently unknown. The groups were closed, with no new introductions, in the twelve months prior to the outbreaks. This coupled with the short, 1 to 4 day, incubation period of CCoV makes the introduction of an infected individual highly unlikely.[3] Domestic dogs may have been a possible source of infection as CCoV type 2 has been shown to be circulating in the domestic dog population in the United Kingdom.[24] A recent report of pantropic canine coronavirus in a wild European wolf in Italy also provides evidence of virus transmission from domestic dogs to exotic canids.[1] The zoological collections where these individuals were housed did not allow domestic dogs on site, reducing the opportunity of direct contact with domestic dogs. However, the seventeen individuals involved in outbreak 1 and 2 were housed in an enclosure on the zoo perimeter, which increased the risk of contact. As transmission usually occurs via the faecal-oral route, typically following exposure to contaminated faeces,[26] fomites, such as tools and footwear, represent another possible source of infection.

No direct link could be found between the 3 outbreaks discussed. However, the authors chose to discuss these cases together to highlight the importance that bush dogs may be highly susceptible to CCoV. With the suspicion of increased susceptibility of this species, enhanced biosecurity of enclosures to minimise the contact between domestic dogs and bush dogs is highly recommended.

Treatment was unsuccessful in severe cases and all individuals died or required euthanasia. Deterioration was usually unexpected and rapid, with no premonitory signs prior to collapse in some cases. In domestic dogs, treatment is limited to supportive care.[20] This can often be initiated earlier in the disease process, as domestic patients are more accessible and amenable to close monitoring, hospitalisation and intensive treatment. Due to the rapid deterioration and lack of response to treatment of the severely affected individuals in this case series, euthanasia on welfare grounds at the time of deterioration is justifiable.

Based on the rapid and fatal disease course of the outbreaks discussed, early diagnosis, quarantine and/or euthanasia of affected individuals should be considered in future cases of CCoV. Infected dogs shed high quantities in faeces, peaking at 4 days post infection.[12] Therefore, the early removal of positive individuals from the group, may limit the levels of virus excreted into the enclosure, reducing the level of challenge experienced by the remaining individuals in the group. Any individuals removed should be barrier managed under strict biosecurity protocols. As shedding has been reported up to six months after infection,[40] individuals should not be returned to the group within this time frame.

Currently, there is no vaccination available for pantropic CCoV. Inactivated vaccines against enteric coronavirus are available in some countries. None of the animals involved in the outbreaks were vaccinated against CCoV and this vaccine is not currently available in the UK. Commercially available vaccines have been shown to be poorly effective against enteric coronavirus.[41] Based on the knowledge that natural immunity against enteric coronavirus is not protective against pantropic coronavirus, vaccines against enteric CCoV are also thought to provide minimal protection.[18] Therefore, preventative measures should be focused on reducing the risk of contact with domestic dogs and fomites.

Conclusions

This case series highlights canine coronavirus as an important enteric pathogen of zoo canids. It should be considered a major differential diagnosis in bush dog groups presenting with severe and fatal diarrhoea. Pathology is typical of viral gastroenteritis but definitive diagnosis requires virus isolation or detection of viral nucleic acid by PCR. Therefore, the collection and storage of faecal material and tissue samples for PCR testing is recommended in suspect cases. Treatment is limited to supportive care and is often unsuccessful. The separation and quarantine of worst affected individuals may aid in the reduction of overall viral load in affected groups. Minimising contact with domestic dogs and fomites is the mainstay of prevention.

Acknowledgments

The author would like to thank the keeping staff and veterinary nurses involved in these cases for their dedication to the care and treatment of the animals.

Primer design

A number of CCV sequences were downloaded from the National Centre for Biotechnology Information (NCBI) database representing CCV Type 1 (AY307021 and KP849472) and Type 2 (DQ112226, GU146061, KP981644, EU856361, EU856362, JQ404409 and AY342160). A set of primers (CCV1_cleave) was designed to amplify a 733 bp segment of the viral genome containing the CCV1 cleavage sites (Forward: 5'-ACTGCTCAAGCTGCTGTAAT-3' and Reverse: 5'-CAACAGTGCCAAGTCCACTA-3'), while a second set of primers (CCV2_cleave) was designed to amplify a 229 bp segment containing the CCV2 cleavage site (Forward: 5'-CCCTTAANTTNGCNTCTGTTG-3' and Reverse: 5'-CCACCNGTACAACGTTTNTAATC-3').

Viral RNA extraction for genotyping

A 100 mg aliquot of faeces was first mixed in 1 mL of 10 M guanidine thiocyanate in Tris HCL containing 3% W/V polyvinylpolypyrrolidone (PVPP) to lyse the faecal matter (All chemicals listed Fisher Scientific, Loughborough, UK). The supernatant from the lysis step was loaded into a Taco™ Automated Nucleic Acid Extraction System (GeneReach, Taiwan) and the purified RNA eluted into 180 µl of elution buffer. This eluate was subsequently used for RT-PCR. PCR reactions consisted of SuperScript III Platinum One Step RT-PCR System reaction mix with dNTPs (Invitrogen, Paisley, UK), nuclease-free water (Qiagen, Manchester, UK) and primers as described below. Each primer (Eurofins MWG Operon, Ebersberg, Germany) was present at a final concentration of 600 nM. Total reaction volumes of 20 µL were used, including 3 µL of template RNA and 17 µL of master mix. The RT-PCR was run on an Eppendorf Mastercycler ep Gradient S (Eppendorf, Stevenage, UK). Cycling conditions were as follows: a reverse transcriptase step at 48°C for 45 minutes, then denaturing at 95°C for 5 minutes, followed by 40 cycles of 95°C denaturation, 56°C annealing and 60°C extension each for 90 seconds followed by a final extension step at 60°C for 10 minutes. PCR products were separated by electrophoresis on an 1.5% agarose gel using a voltage of 120 V for 55 minutes. The CCV1-targeted primers failed to generate a product, while the CCV2-targeted primers produced an amplicon of the expected size. The PCR product was excised from the gel and DNA was extracted using the QiaQuick Gel Extraction Kit (Life Technologies, UK). This purified DNA extract was submitted to Eurofins for PCR-sequencing (Eurofins, MWG Operon), which confirmed the amplified product represented a Type 2 CCV, following identification of the amino acid motif «KRKYRS» in the translated sequence as previously described.31 Following confirmation that the isolate was of Type 2, a set of sub-typing primers19 was used to determine whether the isolate was Type 2a, 2b or 2c CCV. The methodology for the sub-typing assay was the same as previously mentioned, with the exception that the primers were at a final concentration of 300 nM in the reaction mixture and the annealing temperature and cycle timings were slightly adjusted: 40 cycles of 95°C denaturation, 51.7°C annealing, and 60°C extension each for 30 seconds followed by a final extension at 60°C for 10 minutes. A 200 bp product was generated using this method, which corresponds to the CCV 2a subtype.[31]

Figure 4: Complete methodology for the Canine Coronavirus (CCoV) subtyping performed in bush dogs *(Speothos venaticus)*. In order to genotype the strain of CCV present, two novel putatively sub-type specific RT-PCR protocols were developed for CCV1 and CCV2, designed to amplify the S1/S2 and S2 cleavage sites of the respective viral genomes.[31]

Le sous-type 2a du coronavirus canin associé à des épidémies de diarrhée mortelle dans des groupes de chiens des buissons *(Speothos venaticus)*

Trois foyers de diarrhée mortelle sont survenus dans des groupes de chiens de brousse (*Speothos venaticus*) dans deux parcs zoologiques au Royaume-Uni entre 2009 et 2017. Dans tous les cas, les signes cliniques prédominants étaient la diarrhée, l'anorexie et une grave perte de condition. Malgré un traitement de soutien, un certain nombre de décès sont survenus au cours de chaque épidémie. Les résultats macroscopiques courants post-mortem étaient l'émaciation, un érythème, des hémorragies des muqueuses et des ulcération du tractus gastro-intestinal. Les caractéristiques histopathologiques comprenaient un émoussement et une fusion des villosités, une perte épithéliale des cryptes et une déplétion lymphoïde, ce qui confortait une étiologie virale. Un coronavirus canin a été suspecté. Le diagnostic a été confirmé sur la base de la sérologie (augmentation des titres d'anticorps) et de la détection d'acide nucléique viral par amplification en chaîne par polymérase. Le coronavirus canin a été sous-typé comme type 2a, qui est connu pour provoquer une maladie systémique mortelle chez les chiens domestiques immatures. À la connaissance des auteurs, il s'agit des premiers cas signalés de diarrhée mortelle associée au coronavirus canin de type 2a chez les chiens des buissons. Ces épidémies suggèrent que les chiens des buissons adultes sont très sensibles à l'infection par le coronavirus canin et peuvent succomber à une entérite virale.

Mots clés : Canidé, tube digestif, entérite, coronavirus.

Coronavirus canino di sottotipo 2a associato ad un'epidemia fatale di diarrea in gruppi di speoti *(Speothos venaticus)*

Tre focolai di diarrea mortale si sono verificati in gruppi di speoti *(Speothos venaticus)* in due parchi zoologici nel Regno Unito tra il 2009 e il 2017. In tutti i casi, i segni clinici predominanti erano diarrea, anoressia e grave deperimento. Nonostante il trattamento di supporto, un certo numero di decessi si è verificato durante ogni epidemia. I risultati più comuni post mortem erano emaciazione, con eritema, emorragia della mucosa e ulcerazione del tratto gastrointestinale. Le caratteristiche istopatologiche includevano l'ottundimento e la fusione dei villi, la perdita epiteliale della cripta e l'esaurimento dei linfoidi, a sostegno di un'eziologia virale si sospettava il coronavirus canino. La diagnosi è stata confermata sulla base della sierologia (titoli degli anticorpi in aumento) e del rilevamento dell'acido nucleico virale mediante la reazione a catena della polimerasi. Il coronavirus canino è stato sottotipizzato come tipo 2a, che è noto per causare malattie sistemiche fatali nei cani domestici immaturi. A conoscenza degli autori, questi sono i primi casi riportati di diarrea mortale associata al coronavirus canino di tipo 2a negli speoti. Questi focolai suggeriscono che gli speoti adulti sono altamente suscettibili all'infezione da coronavirus canino e possono soccombere all'enterite virale.

Parole chiave: Canide, tratto gastrointestinale, enterite, coronavirus.

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