Review Article

Coronaviruses in Wild Canids: A Review of the Literature

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The Coronaviridae is a ubiquitous viral family, capable of causing disease in domestic and wildlife species, in addition to substantial human disease as indicated by the SARS-CoV-2 pandemic. The spillover of SARS-CoV-2 into wildlife and domestic species is an ongoing concern for both conservation and public health. Following the 2002 SARS-CoV outbreak, surveillance identified a related virus in raccoon dogs that was considered a potential intermediate host between infection in bats and humans. Two other coronaviruses circulate widely in domestic dogs: namely canine coronavirus (CCoV) and canine respiratory coronavirus, and may be transmitted to and amongst wild canids. Domestic dogs have also been identified as an infrequent host for SARS-CoV-2. The spillover of CCoV has been investigated in numerous wild canid populations, primarily using serology. Here, we review reports of coronaviruses in wild canids, helping provide a baseline for future disease surveillance.

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Introduction

The *Coronaviridae* is a family of positive-sense, single stranded RNA viruses (Masters & Perlman 2013). Canine coronavirus (CCoV) is the most widely studied coronavirus of dogs and belongs to the species *Alphacoronavirus I*, which includes two genotypes (or serotypes) called type I and type II (Pratelli et al. 2004, Decaro et al. 2016). Selected examples of the *Coronaviridae* family are depicted as a phylogenetic tree in Figure 1. CCoV is associated with enteric disease, which can range from mild to severe and was first characterized in the 1970s following an outbreak in domestic dogs kept by the military (Binn et al. 1974, Evermann et al. 2005, Decaro & Buonavoglia 2008, Radford et al. 2021). Systemic disease caused by CCoV

 doi.org/10.32388/5UZPYI

remains an open area of investigation (Buonavoglia et al. 2006, Decaro et al. 2008, Zappulli et al. 2008, Pinto et al. 2014). A distinct respiratory virus, canine respiratory coronavirus (CRCoV), has also been identified in domestic dogs and belongs to the betacoronavirus genus, lineage A; it is generally associated with mild clinical signs, though severe disease is possible (Erles et al. 2003). Phylogenetically, CRCoV groups with equine and bovine coronavirus (Figure 1).

The COVID-19 pandemic has raised concerns about SARS-CoV-2 infecting or establishing itself in domestic or wildlife hosts (Leroy et al. 2020). Dogs experimentally infected with SARS-CoV-2 produce neutralizing antibodies in the absence of clinical signs or robust viral shedding (Bosco-Lauth et al. 2020). Several cases of natural SARS-CoV-2 infection in dogs have also been reported (Patterson et al. 2020, Sit et al. 2020). The spread of SARS-CoV-2 into farm-raised mink, initially raised the alarm that SARS-CoV-2 could spread via reverse zoonosis (Munnink et al. 2020). Among the canids, raccoon dogs are farm-raised for fur, have been previously identified to harbor SARS-CoV (Guan, 2003), and are experimentally infectable by SARS-CoV-2 (Freuling et al., 2020). The potential for free-roaming wildlife to be infected with SARS-CoV-2 has now been demonstrated in white-tailed deer (*Odocoileus virginianus)*,which have shown serological evidence of exposure to SARS-CoV-2 (Chandler at al., 2021). Proposed mechanisms by which these may have been exposed include interactions such as hunting, feeding, etc. (Chandler et al., 2021). It is currently unknown if white-tailed deer with SARS-CoV-2 could pose a risk to North American canids that prey upon deer. Lastly, it is possible that wildlife could be exposed to SARS-CoV-2 via wastewater (Franklin & Bevins, 2020), though this also remains unproven. Relevant to SARS-CoV-2 susceptibility is the similarity and therefore binding likelihood with ACE2, the receptor for SARS-CoV-2 entry (Hoffmann et al., 2020). Initial analysis by Damas and colleagues classified the ACE2 of several canids, including the maned wolf (*Chrysocyon brachyuurus*), the dingo (*Canis lupus dingo*), and the bush dog (*Speothos venaticus*) as having a low propensity for binding the ancestral SARS-CoV-2 spike protein (Damas et al., 2020). However, additional analysis has shown the potential for SARS-CoV-2 spike to bind the ACE2 receptor of *Vulpes vulpes* (Luan et al., 2020). Additional work by Zhang and colleagues has demonstrated the importance of specific canine ACE2 residues and SARS-CoV-2 spike for binding (Zhang et al., 2021). Lastly, computer modeling has predicted the order *Carnivora,* to have a high potential for novel coronavirus generation and two Canid species with a higher potential to serves as SARS-CoV-2 hosts included the *Vulpes indica* and *Canis mesomela* (Wardeh et al., 2021).

Highlighting previous coronavirus infections in non-domestic canid species may help in regard to risk analyses, informing surveillance efforts for future studies, understanding the virome in wild canids, and

to drive policy in regards to conservation. Infectious diseases remain a challenge for wildlife conservation (Murray et al. 1999, Daszak 2000). Additionally, urban sprawl along with habitat fragmentation creates challenges for wildlife species, including the higher potential for wildlife to interact with humans and domestic species. The COVID-19 pandemic serves as a reminder of the ability for coronaviruses to jump species. The spread of infectious diseases, including CCoV, from domestic dogs remains a potential concern for wildlife conservation. The risk of domestic dogs to harbor and spread novel coronaviruses is largely unquantified, however, a coronavirus of canine origin, CCoV-HuPn-2018 was previously identified in nasopharyngeal swabs from children with pneumonia (Vlasova et al., 2021). The ability of coronaviruses to recombine is also a concern when considering infections in canids (Decaro et al. 2010). Type II feline coronavirus (FCoV), for instance is considered to have emerged due to a recombination between type I FCoV and canine coronavirus spike genes (Herrewegh et al. 1998, Terada et al. 2014) and type I/type II CCoV recombinants within the spike S1 domain have been identified (Regan et al., 2012). Through a One Health approach, wild canids can be protected from novel viral infections. Mechanisms that can prevent the spread of infectious diseases into wild canid species can minimize the risks of morbidity or mortality from novel coronaviruses and positively impact conservation efforts. From a One Health lens, preventing these novel viral transmission events can prevent potential human pathogen risks. Predicting novel viral spillover risks is challenging; understanding the previous exposure levels observed in wild canids can help define a baseline of exposure and hasten future response efforts when increased disease spillover occurs. Here, we provide a synthesis and review of the literature regarding coronavirus exposure in wild Canidae.

Coronavirus surveillance across genera

Genus Nyctereutes

In 2003, a SARS-like virus was sequenced from a Raccoon Dog (*Nyctereutes procyonoides*) in a live animal market (Guan 2003). Additional surveillance further supported frequent circulation of a SARS-like virus in Raccoon dogs, with 100% of animals considered positive (n=15) utilizing throat and rectal swabs (Kan et al. 2005). In surveillance studies aimed to identify CCoV in farmed raccoon dogs, nearly 92% of animals (22/24) were considered positive for CCoV type II, as assessed via RT-nPCR of feces in addition to a subset of animals (16/24) which were positive for both CCoV type II and type I (Wang et al. 2006). The phylogenetic relationship between the spike protein of coronaviruses identified in raccoon dogs and

other prototypic coronaviruses is shown in Figure 1. Most recently, it has been demonstrated that raccoon dogs are susceptible to experimental infection with SARS-CoV-2 (Freuling et al. 2020). These studies highlight the susceptibility of raccoon dogs to coronaviruses in captive settings. How frequently coronaviruses circulate in non-captive raccoon dogs is unclear, in addition to whether hibernation, for instance, may impact viral loads.

Genus Canis

The circulation of CCoV amongst wolves has been demonstrated in distinct geographical regions (Table 1). An initial survey of wolves (*Canis lupus*) in three areas of Alaska found 213 of 425 to be seropositive and statistical modeling indicated location, season, year and age to be significant predictors of CCoV exposure (Zarnke et al. 2001). More recently, seroprevalence of wolves in Alaska was estimated at 28% and statistical modeling again indicated that seasonality and year were predictors for CCoV seropositivity, suggesting that winter (February to March) transmission is higher (Watts & Benson, 2016). This is similar to a study of Iberian Wolves (*Canis lupus signatus*) in northern Portugal in which viral shedding was suggested to be higher in Autumn and Winter, though there was no statistically significant difference (Rosa et al. 2020). Winter transmission may be due to host factors or viral factors. Amongst several packs of Italian wolves (*Canis lupus italicus)* in Italy and France, the circulation of canine coronavirus was evident via RT-PCR of fecal samples (Molnar et al. 2014). A point the authors do note, however, is the use of RT-PCR targeting alphacoronavirus could be biased, if detecting a coronavirus in a wild boar that was recently consumed (Molnar et al. 2014). In a deceased Italian wolf, CCoV type IIa, considered a pantropic coronavirus, was detected in the gastrointestinal tract, heart, brain, and spleen (Alfano et al. 2019). The cause of death in this wolf remains unknown, as it was also positive for canine parvovirus (CPV) and canine adenovirus-2 (CAV-2) (Alfano et al. 2019). Interestingly, the portion of the coronavirus spike protein that was amplified shared 93% nucleotide identity with a coronavirus previously detected in raccoon dogs (Alfano et al. 2019). Canine parvovirus has also been found to regularly circulate in wolves with CCoV (Molnar et al. 2014, Chitwood et al. 2015, Watts & Benson, 2016, Rosa et al. 2020).

One of the first reports of CCoV in non-domestic canids was in captive coyotes (*Canis latrans*) that were also infected with CPV and consequently developed acute hemorrhagic enteritis (Evermann et al. 1980). A surveillance study of free-range coyotes in North Carolina, USA found a seroprevalence of 32% (9/28) in animals tested between February and June (Chitwood et al. 2015). With the short time frame of testing, it is unknown if coyotes may also experience seasonal changes in seroprevalence similar to that suggested

for wolves in Alaska (Watts & Benson 2016). In a study of captive coyotes, CCoV seroconversion was evident, in addition to 6 of 13 colostrum samples having CCoV antibody with evidence for passive transfer in 20 of 66 pups when tested at 3 days after birth and loss of maternal antibody by 11 weeks of age (n=37) (Green et al. 1984).

Recent SARS-CoV-2 surveillance in coyotes and Eastern wolves (*Canis lupus lycaon*) in Canada did not show evidence of the virus, though only a small number of animals were included (Greenhorn et al. 2021).

Genus Chrysocyon

In a sample of four Bolivian maned wolves (*Chrysocyon brachyurus*), a single animal was serologically positive for CCoV, in addition to being seropositive for CAV, CPV, rabies, and several *Leptospira* serovars (Deem & Emmons 2005). Likewise, while an initial study of seven maned wolves in the Minas Gerais State of Brazil did not detect antibodies to CCoV (de Almeida Curi et al. 2010), a later study found 5 of 11 animals to be seropositive (de Almeida Curi et al. 2012).

Genus Lulupella

In a coronavirus surveillance study utilizing fecal samples from 17 adult silver-backed jackals (*Canis mesomelas*) in Serengeti National Park, one sample was positive and partial sequencing of both spike and membrane proteins showed that it grouped with a type II canine coronavirus (Goller et al. 2013).

Genus Lycaon

In a study of African wild dogs (*Lycaon pictus*) in Kruger National Park, over 60% of animals were serologically positive when tested against an enteric strain of FCoV (WSU79-1683) (Van Heerden et al. 1995). In comparison, a study of African wild dogs in Kenya estimated CCoV seroprevalence closer to 25% (21/83), though seroprevalence declined over the study period between 2001 and 2009 (Woodroffe et al. 2012). Increasing age was associated with seropositivity and surprisingly, coronavirus exposure was not associated with domestic dogs, which had lower seropositivity (Rosie Woodroffe et al. 2012). However, the role of domestic dogs in transmitting coronavirus to African wild dogs remains under investigation and in surveillance studies comparing African wild dogs living in protected versus unprotected areas, the former had lower seroprevalence, though not statistically significant across groups (Prager et al. 2012).

Genus Cerdocyon

In a sample of eight crab-eating foxes (*Cerdocyon thous*) in Brazil, two were considered seropositive for CCoV (Hübner et al. 2010), while two other studies of crab-eating foxes found no evidence for CCoV exposure (de Almeida Curi et al. 2010, Fiorello et al. 2007).

Genus Lycalopex

It remains unclear how commonly Hoary foxes (*Lycalopex vetulus*) are exposed to CCoV, as a sample of two animals, found one to be weakly seropositive in addition to both animals being seropositive for CPV (de Almeida Curi et al. 2010)

In a study including Pampas foxes (*Lycalopex gymnacercus*), three of five animals were considered to have previously been exposed to CCoV based on serology (Hübner et al. 2010), though an additional study in Bolivia did not find serological evidence for CCoV exposure in nine animals (Fiorello et al. 2007).

Genus Urocyon

In Island foxes (*Urocyon littoralis*) sampled in the Channel Islands of California between 2001 and 2003, CCoV seroprevalence was relatively low (3/200) with the positive animals restricted to the island of Santa Catalina, of which 32 animals were surveyed (Clifford et al. 2006). In comparison, a study in 1988 found 60% (12/20) CCoV seroprevalence on the island of Santa Catalina, 7% (2/29) CCoV seroprevalence on Santa Cruz, with all other islands sampled being CCoV seronegative (Garcelon et al. 1992). SARS-CoV-2 testing of a single Canadian Grey fox (*Urocyon cinereoargenteus*) in December 2020 did not detect SARS-CoV-2 (Greenhorn et al., 2021).

Genus Otocyon

In a surveillance study utilizing fecal samples from nine adult Bat eared foxes (*Otocyton megalotis*) in the Serengeti National Park, shedding of alphacoronavirus was not apparent via RT-PCR directed at the S and M genes (Goller et al. 2013). During this investigation, the authors note that no juvenile animals were tested and targeting this group of animals could provide further insight (Goller et al. 2013).

Genus Vulpes

In a recent study, *Vulpes vulpes* were shown to be experimentally susceptible to SARS-CoV-2 (Porter et al., 2022). Surveillance for SARS-CoV-2 has been conducted in a small sample of Canadian red foxes (*Vulpes*

vulpes) between 2020 and January 2021, but did not find evidence of SARS-CoV-2 RNA (Greenhorn et al., 2021). In a small sample of Iberian red foxes (*Vulpes vulpes silacea*) CCoV viral nucleic acid was found in a proportion of samples comprised of spleen and small intestine (Rosa et al. 2020).

Discussion

There is serological evidence for CCoV exposure among several wild canid species and to date, the investigation of SARS-CoV-2 and other coronaviruses in wild canids remains a One Health area of exploration. The use of serology remains helpful for assessing previous exposure to viral infections however, and adapting testing mechanisms developed and validated for domestic dogs can be challenging. Further use of molecular techniques to identify circulating coronavirus variants in canid species is useful for understanding viral transmission and dynamics. The impact of coronaviruses on wild canids remains unclear, including when multiple pathogens are circulating through canid species. Additionally, a major question remains in regards to whether coronavirus infections result in clinical disease in wild canids. Much of the literature to this point has focused on surveillance efforts and creates a challenge understanding the pathogenic potential of these infections. The use of whole genome sequencing, including for animals presenting to wildlife rehabilitation centers, for example, may help elucidate the clinical picture surrounding coronavirus infections in free-roaming canids.

Though a specific plan for controlling CoVs in wild canids may not be necessary, aiming to minimize pathogen spillovers from domestic species into wildlife remains a priority. The identification of SARS-like virus in addition to the susceptibility of this species to SARS-CoV-2, however, necessitates taking a One Health approach, including minimizing human activity that may promote pathogen spillover. Though CC0V is not zoonotic, the potential for unidentified pathogens to spread into humans, from wild canids, including those sold for human consumption does remain a threat and supports rethinking about wildlife farming.

Acknowledgements

We thank the members of the Whittaker Lab, past and present, for their helpful discussions during the preparation of this manuscript. We also thank Jean Millet for critical reading of the manuscript.

AES is supported by NIH Comparative Medicine Training Program T32OD011000.

Figures and Tables

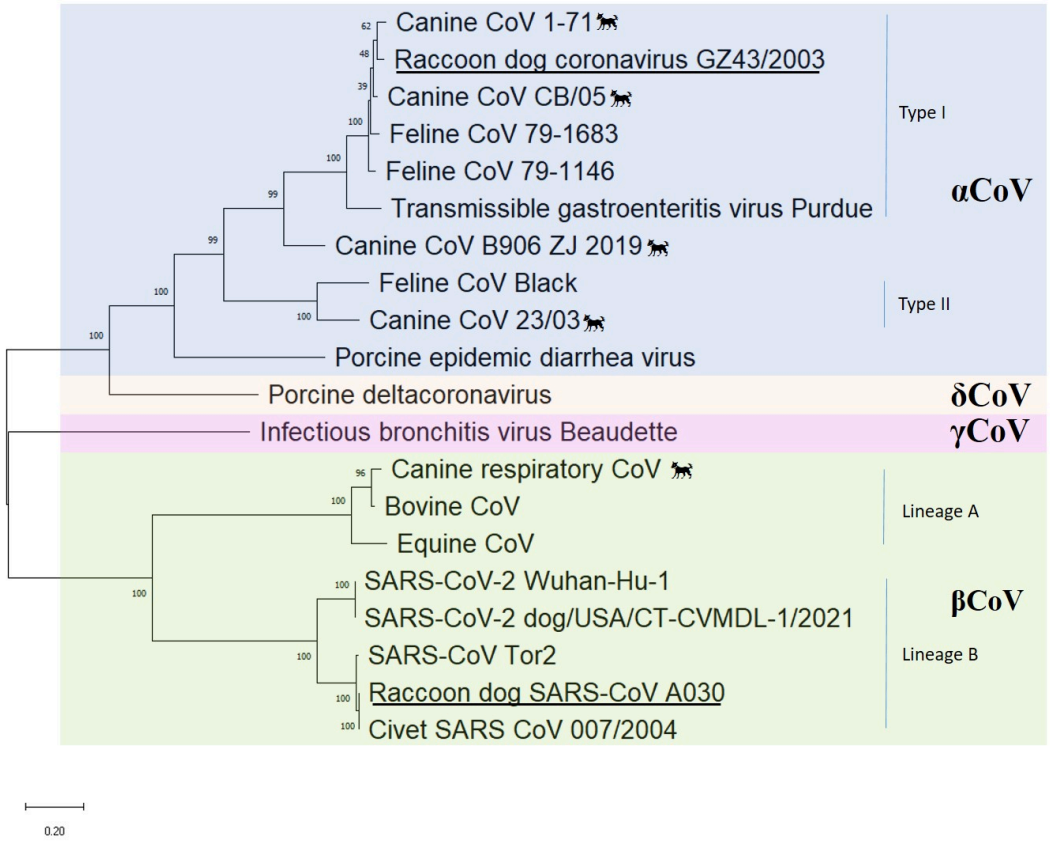

Figure 1. Phylogenetic relationships between CCoV and other coronaviruses based on the spike protein. Prototypic CCoV is classified as an alphacoronavirus. Canine respiratory coronavirus is a betacoronavirus. Underlined, are two viruses that have previously been identified in raccoon dogs. An additional partial spike sequence (HQ339897) has previously been amplified from a silver-backed Jackal, but is not shown, since the sequence was not complete. The phylogenetic tree was made by performing a MUSCLE alignment in Geneious Prime version 2019.2.3. A maximum-likelihood phylogenetic tree was created in MEGAX with bootstrap values based on 1000 replicates (Kumar et al.; 2018). The associated accession numbers are as follows: FCoV 79-1146 (YP_004070194); FCoV 79-1683 (AFH58021); CCoV CB/05 (AAZ91437); CCoV 1-71 (AAV65515); Raccoon dog coronavirus GZ43/2003 (ABO88141); Transmissible gastroenteritis virus (NP_058424); Chinese ferret badger coronavirus DM95/2003 (ABO88142); CCoV B906_ZJ_2019 (QJI07179); FCoV Black (ABX60145); CCoV 23-03 (AAP72150); PEDV (AF500215 1); Asian leopard cat coronavirus Guangxi/F230/2006 (ABQ39958); Infectious bronchitis virus (NP_040831); Canine respiratory coronavirus (VVU96373); Bovine coronavirus (CCE89341); Equine coronavirus (BAS18866); SARS-CoV-2 Wuhan-Hu-1 (YP_009724390); SARS-CoV Tor2 (YP_009825051); Raccoon dog SARS CoV A030 (AAV97987); Civet SARS CoV

007/2004 (AAU04646). SARS-CoV-2 dog/USA/CT-CVMDL-1/2021 was previously detected in a domestic dog and the sequence was obtained after translating the sequence available through the GISAID database (|EPI_ISL_1241386).

Species	Year	Location	Testing method	Results	Reference
Wolf (*Canis lupus*)	1994-1999	Alaska, USA	Serology	213/425	(Zarnke et al. 2001)
	1994-2001	Canada	Serology	0/9	(Philippa et al. 2004)
	2006-2011	Alaska, USA	Serology	28/100**	(Watts & Benson 2016)
Eastern Wolf (*Canis lupus lycaon)*	2020	Ontario, Canada	RT-PCR for SARS-CoV-2	0/5	(Greenhorn et al. 2021)
Italian Wolf (*Canis lupus italicus)*	2005-2006	France	RT-PCR (alphacoronavirus) of feces	4/66*	(Molnar et al. 2014)
	2006-2007	Italy	RT-PCR (alphacoronavirus) of feces	7/79*	(Molnar et al. 2014)
	1995-2011	Portugal	RT-qPCR of Spleen	13/42	(Rosa et al. 2020)
Coyotes (*Canis latrans*)	1972-1982	United States	Serology	12/235	(Foreyt & Evermann 1985)
	1987-1988	Georgia, USA	Serology	0/17	(Holzman et al. 1992)
	1989	Confiscated in South Carolina, USA	Serology	3/13	(Davidson et al. 1992)
	2011	North Carolina, USA	Serology	9/28	(Chitwood et al. 2015)
	2021	Quebec, Canada	RT-PCR for SARS-CoV-2	0/1	(Greenhorn et al. 2021)
Maned wolf (*Chrysocyon brachyurus*)	2000-2003	Bolivia	Serology	1/4	(Deem & Emmons 2005)

Species	Year	Location	Testing method	Results	Reference
	2003-2008	Minas Gerais State, Brazil	Serology	5/11	(de Almeida Curi et al. 2012)
	2004, 2005 (Dry Seasons)	Minas Gerais State, Brazil	Serology	0/7	(de Almeida Curi et al. 2010)
Silver Backed Jackals (*Canis mseomelas*)	2003-2008	Serengeti National Park, Northern Tanzania	RT-PCR (S and M genes, alphacoronavirus) of feces	1/17	(Goller et al. 2013)
African Wild Dog (*Lycaon pictus*)	1988-2009	Botswana	Serology	5/49	(Prager et al. 2012)
	1988-2009	Kenya	Serology	21/86	(Prager et al. 2012)
	1988-2009	South Africa	Serology	5/85	(Prager et al. 2012)
	1988-2009	Tanzania	Serology	0/9	(Prager et al. 2012)
	1988-2009	Zimbabwe	Serology	2/26	(Prager et al. 2012)
	1990-1993	South Africa	Serology	20/31	(Van Heerden et al. 1995)
	1992-1999	Botswana	Serology	13***/106	(Alexander et al. 2010)
	2001-2009	Kenya	Serology	21/83	(Woodroffe et al. 2012)
Crab eating fox (*Cerdocyon thous*)	2001-2005	Bolivia	Serology	0/5	(Fiorello et al. 2007)
	2002-2003	Brazil	Serology	2/8	(Hübner et al. 2010)

Species	Year	Location	Testing method	Results	Reference
	2004, 2005 (Dry Seasons)	Minas Gerais State, Brazil	Serology	0/10	(de Almeida Curi et al. 2010)
Hoary fox (*Lycalopex vetulus*)	2004, 2005 (Dry Seasons)	Minas Gerais State, Brazil	Serology	1/2	(de Almeida Curi et al. 2010)
Pampas Fox (*Lycalopex gymnocercus*)	2001-2005	Bolivia	Serology	0/9	(Fiorello et al. 2007)
	2002-2003	Brazil	Serology	3/5	(Hübner et al. 2010)
Island Fox (*Urocyon littoralis*)	1988	Channel Islands, California, USA	Serology	14/194	(Garcelon et al. 1992)
	2001-2003	Channel Islands, California, USA	Serology	3/200	(Clifford et al. 2006)
Gray Fox (*Urocyon cinereoargenteus*)	1989	Purchased in Indiana, USA	Serology	10/14	(Davidson et al. 1992)
	2020	Quebec, Canada	RT-PCR for SARS-CoV-2	0/1	(Greenhorn et al. 2021)
Bat eared fox (*Otocyon megalotis*)	2003-2008	Serengeti National Park, Northern Tanzania	RT-PCR (S and M genes, alphacoronavirus) of feces	0/9	(Goller et al. 2013)
Red Fox (*Vulpes vulpes*)	1989	Confiscated in South Carolina, USA	Serology	0/46	(Davidson et al. 1992)
	2020-2021	Canada	RT-PCR for SARS-CoV-2	0/11	(Greenhorn et al. 2021)
Iberian Red Fox (*Vulpes vulpes silacea*)	1995-2011	Portugal	RT-qPCR of spleen and small intestine	4/12	(Rosa et al. 2020)

Table 1. Surveillance studies in wild canids. Studies focused on CCoV unless otherwise noted.

*Based on fecal samples, not individual animals

**Authors note some animals were resampled

***Estimated from bar graph

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Declarations

Funding: AES is supported by NIH Comparative Medicine Training Program T32OD011000.

Potential competing interests: The author(s) declared that no potential competing interests exist.