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Zoonoses and Reverse Zoonoses in Wildlife Populations: One Health Approach

Ph.D. thesis
Candidate: Hiba Dakroub
Supervisor: Prof. Danilo Russo

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Dedication

I would like to dedicate this work to the unwavering pillars of my life—my beloved parents, whose boundless love, sacrifices, and encouragement have been the guiding light illuminating my path. To my dear brothers and sisters, your support and belief in my aspirations have been an unending source of strength.

To my niece and cherished nephews, your joyous spirits and innocent laughter have brought immense happiness and motivation during the challenging phases of this journey.

I extend my deepest gratitude to my dear friends, Razane and Mina, whose presence in my life has been a comforting embrace, making me feel at home despite being miles away from my country. Your friendship has been a source of solace and joy throughout this expedition.

Lastly, I dedicate this work to the love of my life, Ali. Your unwavering support and unwavering belief in me, especially during the trials of this past year, have been my anchor and inspiration. Your love has been my refuge, and your encouragement has propelled me forward when the path seemed daunting.

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Abstract

This thesis investigates zoonotic risks in wild mammals in human-dominated landscapes, focusing on coronaviruses and Toxoplasma as models. It aims to establish systematic surveillance to prevent both zoonotic and reverse zoonotic occurrences, emphasizing the One Health framework. The structure includes an introduction, three chapters, and a conclusion. Chapter 1 explores coronaviruses in European bats, assessing spillover risks. Chapter 2 studies SARS-CoV-2 in Central–Southern Italy's bats, examining potential carriers in urban areas. Chapter 3 monitors Toxoplasma gondii in Southern Italy's wild mammals, highlighting its prevalence and advocating for systematic surveillance. The general conclusion synthesizes findings and discusses conservation implications, contributing to informed policies for health, biodiversity, and wildlife management.

Keywords: Surveillance, zoonotic Risks, SARS-CoV-2, molecular Monitoring, *Toxoplasma gondii.*

1. Introduction

In recent decades, the intricate interplay between humans, animals, and the environment has become increasingly evident, illuminating the crucial need for a comprehensive understanding of zoonotic diseases and their transmission dynamics (Wood et al., 2012). Zoonotic diseases, caused by pathogens transmitted to humans from vertebrate reservoirs, take an enormous toll on human health worldwide and had a major impact on human civilization throughout history and have shaped modern societies, governments, and farming practices (Burroughs et al., 2002). While zoonoses represent a public health risk pointed out by the spreading of human infectious diseases emerging from animal reservoirs, reverse zoonoses may cause reduction and even extinction of the wild animal populations susceptible to viruses, which could destroy local biodiversity and ecological balance (Mishra et al., 2021; Anderson et al., 2023).

The growing recognition of zoonotic outbreaks, such as the SARS-CoV-2 pandemic, Ebola, and avian influenza, underscores the critical importance of exploring the key relationships between wildlife, humans, and the intersecting biological, ecological, and societal factors contributing to the emergence, spread, and impact of zoonotic diseases (Mubareka et al., 2023). This necessitates a paradigm shift towards a holistic and integrative approach—One Health—that recognizes the interconnectedness of human health, animal health, and the environment (Queenan et al., 2017).

The One Health approach focuses on collaborative efforts, from multiple disciplines, including human medicine, veterinary science, ecology, environmental science, and public health, to monitor, detect, and control potential zoonotic threats at their source (Erkyihun et al., 2022). It also helps preserve biodiversity, safeguarding public health, and maintaining the ecological balance crucial for the well-being of all living organisms on our planet (Destoumieux-Garzón et al., 2018).

Within the realm of zoonotic diseases, wildlife mammal populations have a significant and multifaceted role, functioning as both reservoirs of the disease and potential hosts for its transmission (Mackenzie et al., 2013). These mammals encompass a diverse range of species, including rodents, bats, primates, and carnivores, among others (Perkins et al., 2005). Due to their close interaction with humans through various interfaces such as urbanization, agriculture, hunting, and deforestation, these mammals can act as vectors or intermediate hosts for pathogens (White et al., 2020). Their natural behaviours, migration patterns, and ecological niches contribute to the maintenance and circulation of zoonotic diseases (Alexander et al., 2012). Notably, some of the most significant disease outbreaks in recent history, including Ebola, SARS, and COVID-19, have been linked with wildlife mammals, and understanding the dynamics of these interactions is crucial not only for disease management and prevention but also for preserving biodiversity and safeguarding global health (Tajudeen et al., 2022; Hussain et al., 2021).

1.1. Coronaviruses and bats

Concentrating on coronaviruses and their correlation with bats as a crucial model for comprehending zoonotic risks within human-dominated settings, this research focuses on bats, acknowledged as reservoirs for various coronaviruses. Their critical role in harbouring these viruses makes them central to this investigation (Bhattacharya et al., 2020).

Bats, constituting one of the most diverse mammalian orders, encompass an extensive array of species occupying various ecological niches globally (Conenna et al., 2021). Their unique physiological and immunological adaptations have intrigued scientists, particularly regarding their association with viruses (Irving et al., 2021). Bats have been identified as reservoir hosts for numerous viruses, including but not limited to coronaviruses, filoviruses, lyssaviruses, and paramyxoviruses (Wang et al., 2019). Their ability to harbor such a plethora of viruses without succumbing to disease challenges conventional paradigms of host-pathogen interactions (Escudero-Pérez et al., 2023). Factors such as their gregarious behavior, long lifespans, high metabolic rates, and immunological peculiarities contribute to their potential as viral reservoirs (Serra-Cobo et al., 2017). Furthermore, their ability to fly, roost in diverse habitats, and cohabitate with multiple bat species facilitates the interspecies transmission and genetic diversity of viruses (Ruiz-Aravena et al., 2022). Understanding the coevolutionary dynamics between bats and viruses is crucial not only for comprehending viral spillover events to other species, including humans and domestic animals, but also for the development of strategies aimed at mitigating the risks of future zoonotic outbreaks stemming from bat-associated viruses (World Health Organization, 2013).

This thesis aims to unravel the prevalence, diversity, and potential risks of coronaviruses spilling over into human populations by meticulously analyzing the virological traits and ecological behaviours of bats. Through an extensive examination involving genetic sequencing and ecological surveys, this study endeavours to shed light on the factors governing the transfer of coronaviruses from bats to humans across species barriers.

1.2. Toxoplasmosis in wild mammals

Examining *Toxoplasma* as a model pathogen, this study investigates the implications and prevalence of toxoplasmosis in wild mammal populations. *Toxoplasma gondii*, an obligate intracellular parasite, stands as a significant zoonotic pathogen capable of infecting warm-blooded animals, including humans (Delegado et al., 2022). Its life cycle unfolds in a complex manner, alternating between definitive hosts, primarily felids, and intermediate hosts, such as mammals and birds (Sullivan et al., 2011). The felids serve as the exclusive hosts where sexual reproduction of T. gondii occurs within the intestinal epithelial cells, leading to the production of oocysts shed into the environment via feces. Upon ingestion by intermediate hosts through contaminated water or food sources, the oocysts transform into rapidly dividing tachyzoites, initiating acute infection. Subsequently, these tachyzoites differentiate into bradyzoites forming tissue cysts, predominantly found in neural and muscular tissues, establishing a chronic phase (Figure 1). This intricate life cycle not only elucidates the parasite's adaptability to diverse environments but also underscores its medical and veterinary importance due to its potential to cause severe complications, especially in immunocompromised individuals and congenitally infected neonates (Brito et al., 2023).

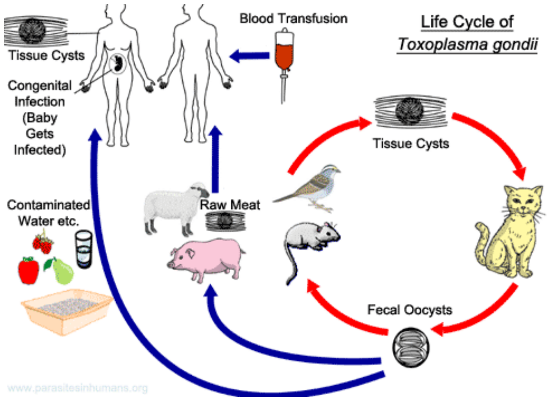

Figure 1. The life cycle of *Toxoplasma gondii* parasite

Understanding the dynamics of *Toxoplasma gondii* transmission among various wildlife species holds significance not only for wildlife health but also for assessing potential risks to human populations (Zhu et al., 2022). Through detailed analyses of environmental factors and host-pathogen interactions, the present study provides information that could be used in future studies that aim to estimate the prevalence of *Toxoplasma gondii* in wild mammals to better understand the parasite's life cycle, its transmission dynamics, and the risk to public health, and to provide useful information for wildlife management and public health protection (Gazzonis et al., 2021).

As the world grapples with unprecedented challenges posed by emerging infectious diseases, this work aspires to offer valuable insights and guide future initiatives towards fostering healthier ecosystems, safeguarding animal, and human well-being, and promoting the coexistence of diverse species on our planet.

1.3. Thesis overview

This thesis thoroughly examines the diverse aspects of investigating zoonotic risks associated with wild mammals in human-dominated landscapes, using coronaviruses and *Toxoplasma* as models for viruses and parasites, respectively. We use coronaviruses and *Toxoplasma*, because they have in common proximity to humans and the potential high probability of interaction. Additionally, it aims to establish systematic surveillance to proactively prevent both zoonotic and reverse zoonotic occurrences that pose threats to human health, biodiversity conservation, and effective wildlife management. This is carried out through the application of a comprehensive One Health framework.

It consists of a general introduction followed by 3 chapters then a general conclusion summarizing the main findings from my research chapters and discussing these results in the context of the conservation implications. Chapter 1 deals with alpha- and beta- coronaviruses circulation In European bats focusing on the epidemiological features that affect the risk of spillover into humans and the possibility of spillback of CoVs from humans to bats. Chapter 2 deals with SARS-CoV-2 circulation in bats of Central–Southern Italy testing the hypothesis that bats occurring in urban areas, or their immediate surroundings, are exposed to SARS-CoV-2 infection and can therefore carry the virus. Chapter 3 deals with molecular monitoring of another zoonotic pathogen, *Toxoplasma gondii,* in wild mammals in Southern Italy. This study indicated the high circulation of *Toxoplasma gondii* in wildlife and remarked on the critical role of anthropized areas where domestic cats and wildlife may come into contact, urging systematic surveillance.

2. Alpha- and beta- coronaviruses In European bats

(Draft)

1 Wildlife Research Unit, Dipartimento di Agraria, Università degli Studi di Napoli Federico II, Portici, Italy
2 Research Institute of Wildlife Ecology, University of Veterinary Medicine Vienna, Vienna, Austria

2.1. Introduction

The incidence of infectious diseases in humans is often caused by pathogens derived from animal hosts, and outbreaks of zoonotic diseases pose a major challenge to global health worldwide (Johnson et al., 2020). In the last two decades, the world has faced three coronavirus (CoV) epidemics and pandemics that caused serious human diseases and considerable global health consequences: the severe acute respiratory syndrome (SARS-CoV) (2002), the Middle East respiratory syndrome (MERS) (2012) and the recent coronavirus disease 2019 (COVID-19) (Guarner, 2020). Until 2003, it was believed that the members of this family only cause mild respiratory illness in humans. However, the emergence of SARS-CoV in 2003 led to extensive research work on these viruses to better understand its origin, zoonotic perspective, and potential intermediate hosts of the virus (Burrell, 2017). Scientists assumed that the COVID-19 virus originated from bats and was transmitted to humans via an intermediate host (Alanagreh et al., 2020). The assumption was manly based on the fact that various related coronaviruses (CoVs) had been circulated in bat populations in China and were isolated from bats before pandemic.

Bats (Order Chiroptera) are an ancient and diverse group of ecologically important mammals, representing nearly a fifth of mammalian diversity, with almost 1400 species recognized (Frick et al., 2020). Fifty-two species represent the diversity of bats in Europe (Kohl & Kurth, 2014). Their ability to fly and migrate, coupled with the ability to navigate at night by using a system of echolocation, living in densely populated colonies, and high genetic diversity have made bats a good candidate for infection and potential transmission of different viruses (Jung & Kalko, 2011). Bats are found worldwide with the exceptions of very high mountains, isolated oceanic islands and extreme polar latitudes (Hutson et al., 2001).

In addition to the important role that bats play in maintaining ecological balance, they have also been speculated to harbour a wide variety of viruses (Banerjee et al., 2019). They have the greatest viral richness among mammals, harbouring a higher proportion of zoonotic viruses than other mammalian taxa (Nabi et al., 2021). Many of these viruses, such as lyssaviruses, filoviruses, henipaviruses and CoVs, can cause disease in humans and many different domestic and wild animal species (Banerjee et al., 2019). CoVs may cause a variety of different diseases in animals thus posing a major threat to livestock and wildlife (Jahangir et al., 2020). For humans, CoVs were thought to cause only mild respiratory infection before the outbreak of SARS-CoV which caused serious human infection and disease. The serious risk that CoVs posed to both human health and wildlife conservation in the last two decades, has increased the importance of bats as a potential reservoir of alpha and beta CoVs.

According to the bat-associated virus database, different viruses have been detected in more than 370 bat species globally (http://www.mgc.ac.cn/DBatVir/; accessed on 17 August 2023) and more than 4800 CoV sequences from 14 bat families have been identified, representing more than 30% of all bat viruses available sequences (Ruiz-Aravena et al., 2022). In Europe, 1613 virus sequences were detected in bats of which more than 440 are CoV sequences (http://www.mgc.ac.cn/DBatVir/; accessed on 17 August 2023).

Thus, the study of bat-associated CoVs has been considered increasingly important to improve our knowledge and understanding of the high biodiversity of bats, and the genetic diversity of bat-associated CoVs.

In this review, we collected information from previous studies of bat CoVs in Europe, including the country, bat species (CoV positive species in bold), sampling type and the year of sampling, detection method detected CoV species and its accession number in GenBank. We summarize the known detections of CoVs in bats (2004-2022) in different European countries and highlight the role of bats as a reservoir host for CoVs. In particular, we focused on the epidemiological features that affect the risk of spillover into humans and the possibility of spillback of CoVs from humans to bats.

2.2. Coronaviruses

Coronaviruses (Subfamily Orthocoronavirinae, family Coronaviridae, order Nidovirales) are a group of large, enveloped, single-stranded, positive-sense RNA viruses with high genetic variability (Poudel et al., 2020). It includes four genera: Alphacoronavirus (α-CoV) and Betacoronavirus (β-CoV), that cause infection in a broad range of mammalian species including humans, and Gammacoronavirus (γ-CoV) and Deltacoronavirus (δ-CoV), which primarily infect birds (Ruiz-Aravena et al., 2022).

CoVs harbor one of the largest genomes among RNA viruses with a size ranging from 27 to 32 kb (Farooq, 2020). Two thirds of their genome are occupied by two genes encoding two large overlapping polyproteins, ORF1a and ORF1b, that encodes a protein containing a conserved RNA-dependent RNA polymerase (RdRp) and other nonstructural proteins involved in RNA synthesis or the modulation of the host response (Fan et al., 2019). The rest of the CoV genomic RNA encodes four structural proteins; spike (S), envelope (E), membrane (M), and nucleocapsid (N), and other accessory proteins (Fan et al., 2019).

Beyond human CoVs, other CoV species cause infection of a broad range of animal hosts (Banerjee et al., 2019), causing a severe infection and even death in some species (Tiwari et al., 2020). Since the first discovery in the 1960s, CoVs caused several outbreaks in different animals such as dogs, cats, pigs, chicken, cows, and camels, causing significant morbidity and mortality (Alluwaimi et al., 2020).

Due to mutations and various viral recombination events in CoVs that have led to the continuous emergence of new viral strains with higher virulence characteristics, there is a high potential of spillover and spread to new host species (Poudel et al., 2020). To date, seven CoVs belonging to alpha- and beta-CoV have been detected that can affect humans. Four of seven CoVs (HCoV-229E, HCoV-NL63, β-CoVs HCoV-HKU1, and HCoV-OC43) have low pathogenicity and may cause mild respiratory symptoms similar to the common cold. The other three CoVs belong to β-CoVs; SARS-CoV (and its recent emergence of SARS-CoV-2), and MERS-CoV, all emerged within the last two decades and led to severe and fatal respiratory tract infections in humans and even some other animal species (Kesheh et al., 2022; Prasad et al., 2020; Sofi et al., 2021).

The capacity of CoVs to enter a cell is mediated by the spike protein, a surface protein which supports both binding to the host cell, through its receptor-binding domain (RBD), and fusion with the membrane of the cell (Ruiz-Aravena et al., 2022). Different host-cell receptors are utilized by viral proteins to recognize host cells, such as integrins, angiotensin-converting enzyme 2 (ACE2), sialic acid receptors, dipeptidyl peptidase 4 (DPP4), and glucose regulated protein 78 (GRP78) (Nassar et al., 2021).

The functional interactions between the viral protein and the host receptor vary according to the host-cell receptor binding affinities. Indeed, the wide host range of several CoVs can be explained by the wide variety of conserved cell receptor structures in different animal species. Thus, direct transmission of CoVs from bats to other mammalian species mainly depends on host-cell receptor structures and the functional binding affinity of the viral RBDs. This is a crucial aspect for characterizing the zoonotic potential of existing bat CoVs (Ruiz-Aravena et al., 2022). (Ruiz-Aravena et al., 2022). The amount and timing of virus shedding by bats are also important in assessing the risk of zoonotic virus transmission. It has been shown that strong and specific replication of RNA viruses occurs mainly during colony formation and after parturition in maternity roosts (Drexler et al. 2011, Joffrin et al. 2022).

2.3. Coronaviruses monitoring in European bats

Since the onset of the SARS pandemic in 2002, the number of monitoring studies on CoVs in bats has significantly increased in various regions of the world, including Europe; as a result, many strains of CoVs have been described in different bat families. Indeed, between 2007 to 2023, more than 30 studies related to bats and CoVs have been conducted in European countries. Bat samples were collected from different locations in 17 different countries in Europe (Figure 2).

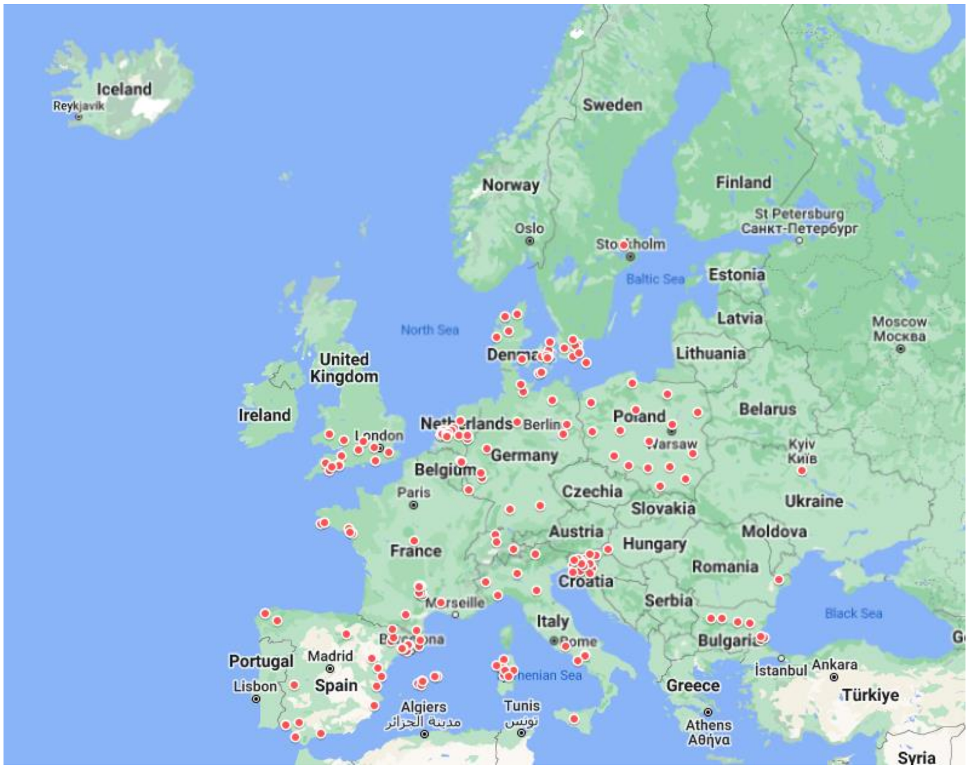

Figure 2. The distribution of the studied bat species collected from Europe. The red dot indicates the location of the sampled bats. A detailed and interactive map of the sample distribution can be accessed via this link: https://www.google.com/maps/d/edit?hl=en&mid=1hQH38F6sw6xfGCGn78a_npyNZ075Y1Q&ll=47.7223872346496%2C11.055929950000008&z=5

The collected samples belonged to 37 bat species (11 families), representing around 70% of bats with known European distributions. These samples were mainly obtained from captured bats before rehabilitation activities, during monitoring and conservation activities, or from bats found dead. The types of samples included oropharyngeal swabs, rectal swabs, and faecal samples, which were collected from live bats or from internal organs from bat carcasses. Faecal samples were either sampled alone or in combination with oral, skin, or rectal swabs, and/or animal carcasses. Two studies out of the 32 included only bat carcasses in their examination.

Twenty-two out of 32 bat CoV monitoring studies were based on PCR assays targeting parts of the RdRp gene, with PCR product sizes ranging from 121 base-pairs (bp) to around 816 bp. Additionally, seven metagenomic studies were performed using Illumina and nanopore sequencing, methods for analysis, and the complete CoV genome data originated from these studies provided some potential characterizations of alpha and beta CoVs isolated from bat samples, as summarized in Table 1.

α- and β-CoV were detected in 29 out of 37 investigated bat species from Europe (Table 1). No CoVs were detected in *Barbastella barbastellus, Myotis mystacinus, M. alcathoe, M. escalerai, M. punicus, Pl. austriacus, Pl. sardus* and *Pl. macrobullaris* species. The results may be explained by the low number of collected samples, therefore those species were underrepresented in monitoring studies.

The diversity of CoVs found in European bats is high (Figure 3). In general, the prevalence of CoVs in bats ranged between 0.4% (Kohl et al., 2021) and 63.2% (Drexler et al., 2011). Such estimates may be influenced by factors such as the geographic regions or sampling sites, the number of species analyzed in the study, the heterogeneity of the sampling sites, the type of samples analyzed, the tests used for sample analysis and their sensitivity and specificity, and finally the number of individual bats sampled.

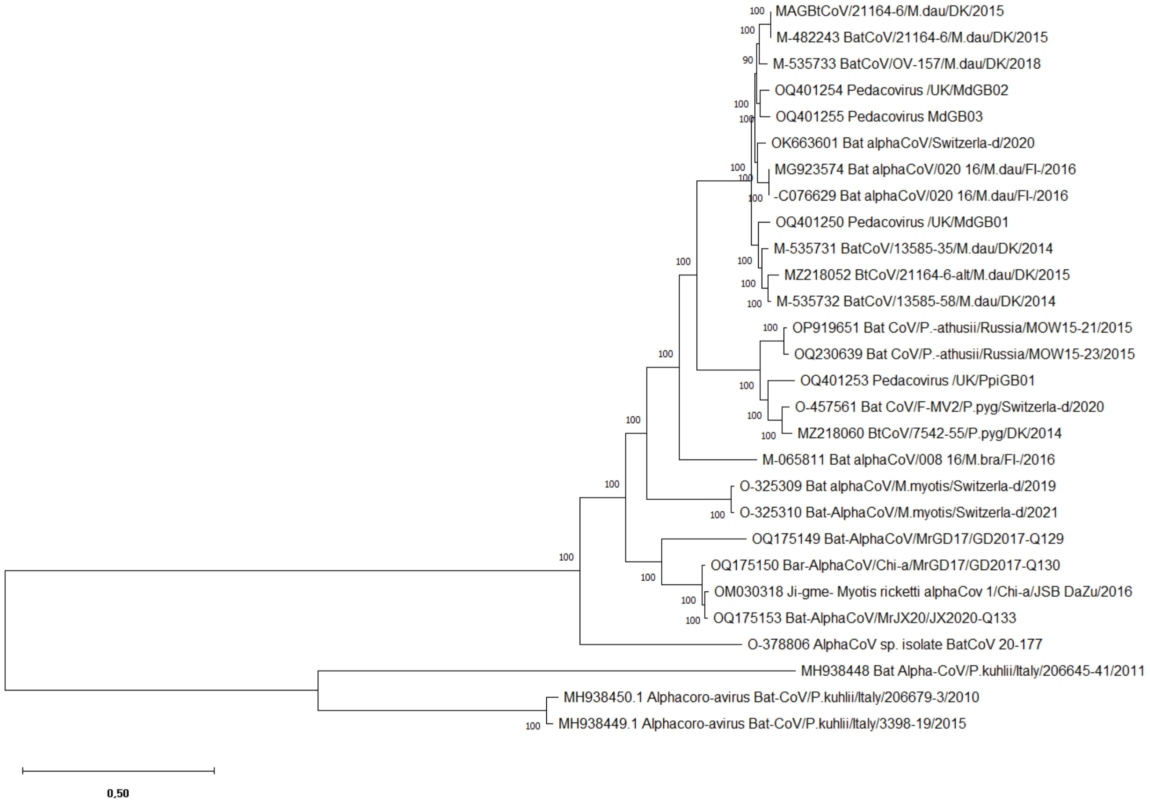

Figure 3. Phylogenetic tree of the full-length genome sequence of European alpha coronaviruses, and wo isolates from China deposited in Genbank. The tree was generated using a maximum likelihood approach (MEGA 11.0 software) based on a GTR + I + G model. The phylogenetic tree was midpoint rooted.

Twenty-eight out of 32 studies sampled faecal materials to search for CoVs. Faecal materials from animals were used to search for CoVs because these viruses may be shed in the feces of infected animals (Kumakamba et al., 2021). Shedding occurs when the virus replicates in the intestinal tract of the infected animal. By analyzing fecal samples, scientists can non-invasively detect and identify viruses present in the animal population, including potential novel strains that may pose a risk to human health (Corman et al., 2014; Wong et al., 2019).

Recent phylogenetic data showed that the SARS-CoV-2, which caused the current COVID-19 pandemic, is not closely related to the CoVs circulated in European bats before and after the pandemic. It is important to investigate the phylogenetic relatedness of SARS-CoV-2 to bat CoVs to estimate their potential to infect humans or other animal species, as the COVID -19 pandemic has demonstrated our gap in knowledge to predict epidemics and pandemics.

In addition to the interactions among the various bat species themselves, interactions between bats and other animals, as well as interactions between bats and human are important for interspecies transmission of viruses (Wong et al., 2019).

The monitoring of coronaviruses in European bats represents a critical area of study due to the potential public health implications and the role of bats as reservoirs for these viruses (Kohl & Kurth, 2014). This review explores diverse monitoring methods, including PCR assays and advanced sequencing technologies like Illumina and nanopore sequencing, highlighting their significance in detecting and characterizing viruses. It also addresses the zoonotic threats posed by these bat coronaviruses, urging continuous surveillance to understand their potential transmission to humans. Overall, this work suggests future research directions to enhance surveillance strategies and gain a comprehensive understanding of coronaviruses in European bats, aiming to mitigate potential risks to both human health and biodiversity.

Table 1. Overview on coronaviruses detected in European bats.

Country		Sampling	Bat species	GenBank accession number	Detection method	Reference
Bulgaria	Elena, Bratanova, Kaleto, Roman, Big Vupa, Malko Tarnovo, Maharata Cave	Faecal samples (2008)	*R. euryale, R. blasii, R. ferrumequinum, R. mehelyi, Mi. schreibersii, N. leisleri, R. hipposideros, M. bechsteinii, B. barbastellus, M. daubentonii, M. emarginatus, M. myotis, P. pygmaeus, M. alcathoe, Pl. auritus, M. nattereri, M. mystacinus, M. oxygnatus, M. capaccini*	α-CoV and β-CoV (GU190215 to GU190248) (130/499, 26%) (Full genome)	Reverse transcription-PCR (RT-PCR) (455 bp)	(Drexler et al., 2010)
Denmark	Vesløs, Mønsted, Bornholm, Lolland, Agerup forest, Funen, Sollerup forest, Vadum, Sorø, Borup, Helsinge,	Faecal samples (2013-2017)	*M. daubentonii, M. dasycneme, M. nattereri, E. serotinus, P. pygmaeus M. bechsteinii, M. mystacinus, M.*	α-CoV *(LR025199-LR025201)* (LR025670-LR025740) (58/271, 21%)	RT-PCR (pan-CoV primers) (Escutenaire et al., 2007, Vijgen et al., 2008) Metagenomics	(Lazov et al., 2018)

	Tureby, Tim, Sakskøbing		*brandtii, N. noctula, Pl. auritus,*		(130 bp, 208 bp and 381 bp)	
	Mønsted, Sollerup, Vadum	Faecal samples (2014-2016, 2018)	*M. daubentonii, M. dasycneme,* *P. pygmaeus*	α-CoV (MN535731, MN535732, MN543743, MZ218052, MN535733, MN535734, MN482242, MZ218060) Full genome	Illumina	(Lazov et al., 2021)
Finland	South-central Finland	Faecal samples (2014-2016)	*E. nilssoni, M. brandti, M. daubentonii, P. nathusii, Pl. auritus, Unidentified species*	α-CoV and β-CoV *(*MG923567-MG923574) (MN065811) (10/79, 13%)	RT-PCR, RT-qPCR, and NGS (440 bp)	(Kivisto et al., 2020)
France	Laissac, Lagarde, Dions, Lapanouse, Pontchâteau, Cantoin, Batère, Pluherlin, Plovan, Lacalm, Cruéjouls, Creissels, Brénac, La Roche Bernard, Biounac, Quimper, Orlhaguet, Sainte-Geneviève-sur-Argence	Faecal samples (2008-2016)	*Mi. schreibersii, R. ferrumequinum, E. serotinus, M. daubentonii, M. nattereri, R. hipposideros, B. barbastellus, M. alcathoe, M. bechsteinii, M. blythii, M. capaccinii, M. emarginatus, M. escalerai, M. myotis, M. mystacinus, M. nattereri_ssp.a (new species), P. pipistrellus, P. pygmaeus, Pl. austriacus*	α-CoV and β-CoV (KY423375-KY423491) (161/1179, 13.6%)	semi-nested PCR (121 to 393 bp)	(Ar Gouilh et al., 2018)
France	Bourges	Faecal samples (2014)	*P. pipistrellus, B. barbastellus, M. myotis, E. serotinus*	α-CoV (KT345294-KT345296) (5/162, 3.1%)	nested reverse transcription (RT)-PCR souza et al., 2007	(Goffard et al., 2015)

Germany	Segeberg	Faecal samples (2007)	*M. bechsteinii, M. dasycneme, M. daubentonii, P. nathusii, P. pygmaeus, M. brandtii, N. noctula*	type I CoVs (31/315, 9.8%)	Nested reverse transcription–PCR (RT-PCR) (De souza et al., 2007) (440-bp)	(Gloza-rausch et al., 2008)
	Rhineland-Palatinate	Faecal samples (2008-2010)	*M. myotis*	α-CoV (HM368166) (297/470, 63.2%)	reverse transcription–PCR (RT-PCR) (455 bp)	(Drexler et al., 2011)
	North Rhine Westphalia, Bavaria, Mecklenburg Western Pomerania	Faecal samples (2011-2014)	*M. nattereri, P. nathusii, P. pygmaeus, M. bechsteinii, M. daubentonii, Pl. auritus, P. pipistrellus*	α-CoV (KT894921 - KT894926) (11/653, 1.7%)	Nested reverse transcription–PCR (RT-PCR) (souza luna et al., 2007)	(Fischer et al., 2016)
	Bavaria, Lower Saxony, Berlin greater metropolitan area, Brandenburg, Baden Wuerttemberg	Intestinal sample	*P. pipistrellus, M. mystacinus, M. daubentonii, M. bechsteinii, M. brandtii, M. myotis, M. nattereri, P. nathusii, P. kuhlii, P. pygmaeus, V. murinus, B. barbastellus, Pl. austriacus, Pl. auritus, E. nilssonii, E. serotinus, N. noctula, N. leisleri*	α-CoV of group 1 MN851285 (1/240, 0.4%)	CoV PCR (de souza luna et al.)	(Kohl et al., 2021)
Hungary	Allover Hungary	Faecal samples (2012-2013)	*R. Euryale, R. ferrumequinum, R. hipposideros, M. daubentonii, M. myotis, M. nattereri, P. pygmaeus, B. barbastellus, E. serotinus, Mi. schreibersii, M. alcathoe, M. bechsteinii, M. brandtii, M. dasycneme, M. emarginatus, M. mystacinus, M. blythii, N. leisleri, N. noctule, P. nathusii, P. pipistrellus, Pl. auritus,*	SARS-relatedβ, α-CoV KJ652335 (8/447, 1.8%)	nested reverse transcription (RT)-PCR souza et al., 2007	(Kemenesi et al., 2014)

			Pl.austriacus, V. murinus			
Italy	Borutta, S.M. Coghinas, Ula-Tirso, Dorgali, Bonorva, Bolotana, Belvì, Alghero	Oral, skin swabs and fecal samples. (2015-2016)	*R. ferrumequinum, Pl. auritus, T. teniotis, R. mehelyi, Mi. schreibersii, M. punicus, M. capaccinii, M. emarginatus, Pl. sardus, H. savii, P. pipistrellus, P. kuhlii, Pl. austriacus, N. leisleri, E. serotinus*	β-CoV (MG975784) (3/46, 6.5% total species)	Nested PCR (modifies from Balboni et al., 2023) (170 bp and 440 bp)	(Lecis et al., 2019)
	All over peninsular Italy	Anal swabs and fecal samples (2009)	*R. ferrumequinum*	β-CoV (893/09-11) (2/52, 3·8% total species)	PCR 440 bp	(Balboni et al., 2011)
	Piedmont, Liguria	Saliva, urine and faecal samples (2013-2016)	*P. kuhlii, P. pipistrellus, M. myotis, M. nattereri, M. oxygnathus, Pl. auratus, R. ferrumequinum, P. nathusii, M. brandtii, M. bechsteinii, M. daubentonii, M. emarginatus, M. mistacinus, H. savii, Pl. austriacus, Pl. macrobullaris, B. barbastellus, N. leisleri, R. hipposideros*	15 α-CoV and 5 β-CoV. (KY780381-KY780400) (36/302, 11.9%)	Semi nested PCR (Poon et al, 2005) (537 bp)	(Rizzo et al., 2017)
	Lombardy and Emilia Romagna	Faecal and carcass samples (2010-2012)	*P. kuhlii, H. savii, N. noctula, R. hipposideros, P. spp., T. teniotis, Pl. auratus, V. murinus*	α-CoV and β-CoV (KF500940-KF500953) (16/195, 8.2% total)	pan- CoV (385 bp)	(Lelli et al., 2013)

	Emilia Romagna	Bat carcasses (intestine) (2011)	*H. savii, P. kuhlii*	MERS-like clade 2c (MG596802 and MG596803) (2 out of 2 full genome study)	pan- CoV one-step RT-PCR (Lelli et al., 2013) (180 bp) NGS (Full genome)	(Moreno et al., 2017)
	Emilia Romagna	Two Faecal samples (2010 and 2015) and one carcasses (intestine) (2010) were taken for whole genome sequencing	*P. kuhlii*	(MH938448, MH938449, MH938450	NGS (Full genome)	(De Sabato et al., 2019)
	Sicily, Lombardy, Trentino-south tyrol	Anal swabs (2009-2012)	*M. blythii, E. serotinus, M. myotis*	α-CoV and β-CoV (KF312400, KF312399) (2/75, 2.6%)	nested RT-PCR (De souza et al., 2007)	(De Benedictis et al., 2014)
	Frosinone, Benevento, Naples	Saliva and faecal samples (2021)	*Miniopterus schreibersii, Myotis capaccinii, Myotis emarginatus, Myotis crypticus, Nyctalus leisleri, Pipistrellus kuhlii, Pipistrellus pipistrellus, Hypsugo savii, Rhinolophus ferrumequinum, Rhinolophus euryale*		RT-PCR (Drosten et al., 2003) and (Amoroso et al., 2020) (251 and 452 bp)	(Dakroub et al., 2022)
Luxembourg	Bech-Kleinmacher	Faecal samples (2015-2016)	*M. emarginatus, R. ferrumequinum*	α-CoV and β-CoV (KY502384-KY502388-KY502395) (43/878, 4.9%)	Nested PCR (Chu et al., 2011) (555 and 602 bp)	(Pauly et al., 2017)
Netherlands	Tollebeek, Lisse, Stokkelaarsbrug, Almere, Noordwijk aan Zee, Noordwijkerhout, Wassenaar,	Faecal samples (2009)	*P. pipistrellus, M. daubentonii, M dasycneme, N. noctule, E. serotinus, M. bechsteinii, M. brandtii, M.*	Group 2 CoV Group 1 CoV (GQ259960-GQ259977) (35/211, 16.6%)	nested CoV genus-wide reverse transcription (RT-PCR) De souza luna et al., 2007	(Reusken et al., 2010)

	Scheveningen, Stompwijk, Den Haag, Rijswijk, Leidschendam, Waverveen, s' Gravenland, Papenveer, Ter Aar, Woerdense verlaat, Reeuwijk, Gouda, Doetinchem, Veldhuizen, Zeddam, Westrand, Meerssen, Berg en Terblijt, Geulhem, Sibbe.		*emarginatus, M. myotis, M. mystacinus, M. nattereri, P. nathusii, Pl. auritus*			
	NOT MENTIONED	Bat carcasses (2016-2021)	*P. nathusii*	β-CoV (OQ348392-OQ348406) (OQ405399, OQ405401) (25/88, 28%)	PN-bCoV Up-E gene and MERS-CoV Up-E gene via RT-qPCR For sequence nested pcr Luna at al., 2007	(Mols et al., 2023)
Poland	Mazovian, Lower Silesia, Greater Poland, Silesia, Pomerania, Łódź, Lesser Poland, West Pomerania, Lubusz, Kuyavian-Pomeranian, Opole, Podlaskie, Świętokrzyskie, Warmian-Masurian, Subcarpathian, Lublin.	Oral and faecal samples (2020-2022)	*M. daubentonii, R. hipposideros, E. serotinus, N. noctule, N. leisleri, B. barbastellus, Pl. auritus, V. murinu, P. pygmaeus, P. pipistrellus, P. spp., P. nathusii, M. dasycneme, M. myotis, M. mystacinus, M. nattereri, M. bechsteinii, M. alcathoe, M. brandtii, M. emarginatus, unidentified*	β-CoV *sars-like* (ON873767, ON873769, ON873770, ON873766 and ON873768) (20/503, 4% pos)	(rtRT-qPCR) Corman et al., 2020 Nested PCR by De souza luna et al., 2007	(Orłowska et al., 2022)
Netherland, Germany, Romania, Ukraine	Gelderland (Neth), Rhineland-Palatinate (Ger), Schleswig-Holstein (Ger), Tulcea (Rom), Kiev (Ukr)	Faecal samples (2009-2011)	*P. nathusii, P. pipistrellus, P. pygmaeus, P. kuhlii*	β-CoV (KC243390-KC243392) (40/272, 14.7%)	nested reverse transcription PCR (RT-PCR)	(Annan et al., 2013)

Slovenia	Kočevje, Slovenska Bistrica, Loška dolina, Postojna, Vnanje Gorice, Verd, Goče, Krašnja, Volovščica, Trebnje, Dolnji Suhor, Kobiljača, Završe, Kobilje, Mali Obrh, Močnik, Račna, Škocjan, Puščava, Dobrova, Selca, Snežnik, Velike Lašče, Iška vas, Cerkno, Rižana	Faecal samples (2008)	*R. hipposideros, M. daubentonii, M. mystacinus, M. myotis, P. kuhlii, P. nathusii, E. serotinus*	Group 2 of CoVs (GQ404795-GQ404797) (14/106, 13.2%)	RT-PCR (Stephensen et al., 1999)	(Rihtarič et al., 2010)
Spain	Sant Llorenç Savall, Olesa de Bonesvalls, Inca, Majorque, Malgrat de Mar, Os de Balaguer, Rocafort, Palma, Majorque, Oliete, Ferreries, Llucmajor, Majorque, Llimiana, Orpi, Senet, Santa Maria de Miralles, Vilafranca	Faecal samples (2008-2016)	*Mi. schreibersii, T. teniotis, R. ferrumequinum, M. capaccinii, M. myotis, E. serotinus, H. savii, M. blythii, M. daubentoniid, M. escalerai, N. leisleri, P. kuhlii, P. pipistrellus, Pl. austriacus*	α-CoV and β-CoV (KY423375-KY423491) (46/347, 13.2%)	semi-nested PCR (Gouilh et al., 2011)	(Ar Gouilh et al., 2018)
	Coruña, Lugo, La Rioja, Gerona, Castellón, Valencia, Alicante, Málaga, Cádiz, Sevilla, Huelva, Cáceres, Menorca	Faecal samples and Oro-pharyngeal swabs (2004-2006)	*E. isabellinus, H. savii, Mi. schreibersii, M. daubentonii, M. myotis, M. blythii, N. lasiopterus, P. kuhlii, P. sp., B. barbastellus, E. serotinus, M. alcathoe, M. bechsteinii, M. capaccinii, M. emarginatus, M. escalerai, M. mystacinus, M. nattereri, N. leisleri, P. pipistrellus, P. pygmaeus, Pl.*	α-CoV and β-CoV (HQ184049-HQ184062) (Total 14/576, 2.4%)	pan- CoV nested PCR (512 bp)	(Falcón et al., 2011)

			auritus, Pl. austriacus, R. euryale, R. ferrumequinum, R. hipposideros, R. mehelyi			
Sweden	Hammarskog	Saliva swabs and faecal samples (2020)	*P. pygmaeus*	α-CoV (ON457560-ON457562)	Illumina	(Cholleti et al., 2022)
	Vramsån, Verkaån, Nybroån, Bråån, Everöd, Simrishamn, Vinslöv	Faecal samples (2020)	*M. daubentonii, P. pygmaeus, M. mystacinus*	α-CoV (OK663601) (1/16, 6.3%)	Next-generation sequencing	(Lwande et al., 2022)
Switzerland	Aargau, Grisons, Lucerne	Faecal samples (2019-2021)	*V. murinus, M. myotis, R. hipposideros*	Aphacov and MERS-related CoV (MN535733) (ON325307–ON325310) (full genome)	Next generation sequencing (Illumina)	(Wiederkehr et al., 2022)
United Kingdom	Wytham, Savernakeb, Southwest England	Faecal sample (2006-2009)	*M. nattereri, M. daubentonii, P. auritus, R. ferrumequinum, R. hipposideros, P. pipistrellus, B. barbastellus*	α-CoV (JF440349-JF440366) (26/112, 23.2%)	Semi-nested PCR (de Souza Luna 2007)	(August et al., 2012)
	Somerset, Monmouthshire, Wales	Faecal samples (2020)	*R. hipposideros*	RhGB01 has a 77% (SARS-CoV-2) and 81% (SARS-CoV) amino acid homology (1/53, 1.9%) (full genome)	Metagenomics	(Crook et al., 2021)
	Colyton, Buckfastleigh, Chudleigh, West Sussex, Kent, West hatch	Faecal samples (2020-2021)	*M. daubentonii, P. pipistrellus, P. pygmaeus, Pl. auritus, R. ferrumequinum, R. hipposideros, M. nattereri, M.*	α-CoV and β-CoV (OQ401247-OQ401251, and OQ401253-OQ401255)	Metagenomics	(Tan et al., 2023)

			bechsteinii, E. serotinus, M. mystacinus, P. nathusii, N. noctule, M. brandtii, B. barbastellus, N. leisleri, M. alchathoe	(9/48, 18.75%) (full genome)		

B= Barbastella, E= Eptesicus, H= Hypsugo, M= Myotis, Mi= Miniopterus, N= Nyctalus, P= Pipistrellus, Pl= Plecotus, R= Rhinolophus T= Tadarida, V= Vespertilio

3. A First Assessment of SARS-CoV-2 Circulation in Bats of Central–Southern Italy

Hiba Dakroub [1,2] , Danilo Russo [1,†] , Luca Cistrone [3], Francesco Serra [2], Giovanna Fusco [2], Esterina De Carlo [2] and Maria Grazia Amoroso [2,,†]*
1 Wildlife Research Unit, Dipartimento di Agraria, Università degli Studi di Napoli Federico II, Via Università, 100, 80055 Portici, Italy
2 Department of Animal Health, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Via Salute,2, 80055 Portici, Italy
3 Forestry and Conservation, Via Botticelli, 14, 03043 Cassino, Italy
* Correspondence: mamoroso@izsmportici.it; Tel.: +39-081-786-5116
† These authors contributed equally to this work.
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3.1. Abstract

One serious concern associated with the SARS-CoV-2 pandemic is that the virus might spill back from humans to wildlife, which would render some animal species reservoirs of the human virus. We assessed the potential circulation of SARS-CoV-2 caused by reverse infection from humans to bats, by performing bat surveillance from different sites in Central–Southern Italy. We restricted our survey to sampling techniques that are minimally invasive and can therefore be broadly applied by non-medical operators such as bat workers. We collected 240 droppings or saliva from 129 bats and tested them using specific and general primers for SARS-CoV-2 and coronaviruses, respectively. All samples (127 nasal swabs and 113 faecal droppings) were negative for SARS-CoV-2, and these results were confirmed by testing the samples with the Droplet Digital PCR. Additionally, pancoronavirus end-point RT-PCR was performed, and no sample showed specific bands. This outcome is a first step towards a better understanding of the reverse transmission of this virus to bats. Although the occurrence of a reverse zoonotic pattern can only be fully established by serological testing, the latter might represent an in-depth follow-up to a broad-scale preliminary assessment performed with our approach. We encourage the systematic surveillance of bats to help prevent reverse zoonotic episodes that would jeopardize human health, as well as biodiversity conservation and management.

Keywords: bats; coronaviruses; SARS-CoV-2; spillback; zoonotic viruses

3.2. Introduction

In late December 2019, cases of pneumonia with unknown aetiology were reported in the city of Wuhan, China (Islam et al., 2021). The causative agent, identified as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), is closely related to SARS-CoV, which was responsible for the 2003 SARS outbreak (Al-Qahtani et al., 2020). SARS-CoV-2 caused a sizable epidemic of coronavirus disease 19 (COVID-19) in China, which spread globally and was declared a pandemic in March 2020 (Krammer et al., 2020). At the time of writing, there are 476,374,234 confirmed cases of COVID-19, including 6,108,976 deaths, according to the World Health Organization (WHO) (https://COVID19.who.int) (accessed on 26 March 2022).

SARS-CoV-2 belongs to the subfamily Coronavirinae, family Coronaviridae, order Nidovirales (Kenney et al., 2021). The evolutionary origin of SARS-CoV-2 is still unknown, but it is most probably zoonotic since the virus is approximately 79% similar to SARS-CoV, which is found in wildlife (Holmes et al., 2021). Moreover, SARS-CoV-2 shares a high level of genetic similarity (96.3%) with the bat coronavirus RaTG13 (Zhai et al., 2020), which was isolated from bats in Yunnan in 2013; however, none of the known bat SARS-CoVs are thought to be the immediate ancestor of SARS-CoV-2 (Hu et al., 2017).

The possible causes of the epidemiological origin of SARS-CoV-2 are unclear but the spillover is suspected to be linked to the consumption of wildlife. Based on epidemiological data, the outbreak originated in the city of Wuhan in Hubei Province of central China (Yuen et al., 2020). Two of the three earliest documented COVID-19 cases were directly linked to a market selling wild animals, as were 28% of all cases reported in December 2019 (Holmes et al., 2021).

While the epidemiological route that led to the spillover is far from being fully understood, one serious concern associated with this pandemic is that the virus might spill back from humans to wildlife, which could render some animal species reservoirs of the human virus. This would be especially problematic if the species in question are threatened because their correct management would be jeopardized by their role as a reservoir. Reverse zoonoses pose serious risks to both human health and wildlife conservation (Fagre et al., 2022), and, with reference to the COVID-19 pandemic, this topic has received a great deal of attention (Gryseels et al., 2021). Recently, a joint statement by the Food and Agriculture Organization (FAO), the World

Organisation for Animal Health (OIE), and the World Health Organization (WHO) highlighted that, although COVID-19 is a human pandemic, the virus may infect other animal species besides humans, and reverse zoonoses may affect the health of animal populations, promoting the evolution of new viral variants (https://www.who.int/news/item/07-03-2022-joint-statement-on-the-prioritization-of-monitoring-sars-cov-2-infection-in-wildlife-and-preventing-the-formation-of-animal-reservoirs#:~:text=Current%20knowledge%20indicates%20that%20wildlife,emergence%20of%20new%20virus%20variants, accessed on 18 April 2022). On such bases, the statement emphasizes the importance of monitoring mammalian wildlife populations for SARS-CoV-2 infection.

Although SARS-CoV-2 has never been observed in bats to date, viruses that are highly similar to SARS-CoV2 have been isolated in these mammals (Temmam et al., 2022): it is, therefore, legitimate to hypothesize that bats are more exposed than other species to being infected by humans. This is especially true since many bat species roost in urban areas, where they share buildings with humans (Russo et al., 2015), fall victim to domestic cats, which might provide an epidemiological link (Salinas-Ramos et al., 2021), and are handled by bat rehabilitators or researchers (Medellin et al., 2020; Nunez et al., 2020). Despite experimental research failing to prove a significant risk of SARS-CoV-2 reverse zoonosis involving bats (Schlottau et al., 2020; Hall et al., 2021), studies on captive subjects do not fully replicate the epidemiological dynamics and environmental conditions that occur in the wild, so their outcomes warrant prudence (Fagre et al., 2022). A risk assessment exercise of SARS-CoV-2 transmission from humans to bats carried out in Australia concluded the risk to be low yet with a high degree of uncertainty (Cox-Witton et al., 2021). To date, the number of published studies with surveillance results of SARS-CoV-2 circulation in bat populations is still small (Wacharapluesadee et al., 2021; Jemersic et al., 2021; Saeed et al., 2021). Preventing SARS-CoV-2 reverse zoonosis from humans to bats is paramount not only due to human health concerns but also because many bat species are at risk (Frick et al., 2020) and losing bats would mean losing the crucial ecosystem services they deliver in farmland, forests, and urban areas (Kunz et al., 2011).

With an awareness of the importance of bat surveillance to monitor the potential risk of SARS-CoV-2 reverse infection from humans to bats, our general objective is to contribute to filling the existing knowledge gap on this topic. We test the hypothesis that bats occurring in urban areas, or their immediate surroundings, are exposed to SARS-CoV-2 infection and can therefore carry the virus. Although unambiguous, full evidence of a reverse zoonotic pattern can only be provided by a serological analysis coupled with a virus search in droppings and/or saliva. In this preliminary assessment, we adopted the method used in previous studies, such as those conducted in the UK (Jones et al., 2022). Therefore, we conducted a virus search only to specifically explore the hypothesis that bats will shed the virus in a period of high viral circulation in the study area (from May to July 2021). The advantage of this method is that it can be applied on a large geographic scale by bat operators not trained specifically to take blood samples and routinely incorporated in all bat surveys due to its very limited invasiveness. Sampling bat blood in several countries requires special training (Jones et al., 2022) or is restricted to medical professionals only, as is the case in Italy. On the other hand, while serological analyses conclusively show the existence of contact between the virus and bat populations, blood sampling in small mammals of protected species such as bats requires specific authorization and training of operators (in some countries, this can only be carried out by medical professionals).

3.3. Materials and methods

3.3.1. Sampling

In 2021, we sampled 129 bats in different periods, i.e., May (31 bats), June (45 bats), and July (53 bats), from the Campania and Lazio regions (Central–Southern Italy) at drinking or foraging sites and near roosts with mist nets and harp traps. We collected samples from 11 bats from the province of Frosinone (Lazio), 22 from the province of Benevento (Campania), and 96 from the province of Naples (Campania). For each bat, we measured forearm length (mm) with a 0.1 precision calliper and body weight with a 0.1 g precision weigher. We established the species following Dietz et al. (2009) (Dietz et al., 2009) and ascertained sex, reproductive status (Racey et al., 2009), and age class (Wilkinson et al., 2009). Each bat was kept in clean cotton bags for ca. 20 min before being processed, and all droppings in the bag were collected and stored in sterile vials. Saliva samples were collected with sterile swabs. All samples were immediately brought to the laboratory in a refrigerated box. Field operators tested negative for SARS-CoV-2 by molecular assay before going into the field. Moreover, the operators strictly followed the guidelines established by the IUCN (Nunez et al., 2020) and took all precautions to avoid accidental transmission of SARS-CoV-2 to the bats they handled as well as any pathogen transmission from handled bats to humans.

3.3.2. Nucleic Acid Extraction

Samples underwent nucleic acids extraction by the MagMax^{TM} Viral/Pathogen II Nucleic Acid Isolation Kit (Applied Biosystems, Waltham, MA, USA) following the manufacturer's instructions. Before extraction, oro-pharyngeal swabs were suspended in 0.6 mL of phosphate-buffered saline (PBS) and incubated at room temperature for 30 min. Droppings were suspended in 0.9 mL PBS, vigorously vortexed for 3 min, centrifuged at 13,000 rpm for 3 min, and 0.6 mL of supernatant was transferred to a clean Eppendorf tube. Then, 20 µL of protease k was added, and samples were incubated for 10 min at 70 °C. Next, 200 µL of pre-treated samples was loaded on the MagMax extraction plate, and 5 µL of internal control (included in TaqPath^{TM} COVID-19 RT-PCR kit) was added to each sample before extraction to check the quality of the extraction.

Extracted nucleic acids were eluted in 80 µL elution buffer and immediately analysed by Real-Time RT-PCR/RT-PCR or stored at −20 °C until further processing.

3.3.3. Real-Time RT-PCR for the Detection of SARS-CoV-2

The presence of SARS-CoV-2 was established by Real-Time RT-PCR using the TaqPath^{TM} COVID-19 RT-PCR kit (Thermo Fisher Scientific, Waltham, MA, USA). In the process, probes anneal to three target sequences that are specific to SARS-CoV-2. Each target is located between unique forward and reverse primers for the following genes: ORF1ab, N protein, and S protein.

The reaction was carried out following the manufacturers' instructions. To analyse and interpret the data, we employed the Applied Biosystems™ COVID-19 Interpretive Software running into a QuantStudio™ 5 Real-Time PCR Instrument, 0.1 mL block (Applied Biosystems). Positive and negative controls were included in the kit. SARS-CoV-2 Delta variant and Omicron variant extracted from human swab samples (confirmed as positive by sequencing) were used as supplementary positive controls.

3.3.4. SARS-CoV-2 Droplet Digital PCR

To double-check for the presence of SARS-CoV2, we also carried out a ddPCR using the QX200 Droplet Digital PCR System with the One-Step RT-ddPCR Advanced Kit for Probes (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. The pre-amplification mixture was prepared using 5.5 µL supermix, 2.2 µL reverse transcriptase, 1.1 µL of 300 mM DTT, and 1.25 µL TaqPath^{TM} COVID-19 RT-PCR mix, and 5.5 µL of sample template in a final volume of 22 µL. Each reaction mix was converted to droplets with the QX200 droplet generator (Bio-Rad), sealed for 5 s at 180 °C, and pre-amplified under the following cycling protocol: 50 °C for 60 min, 95 °C for 10 min, then 8 cycles of 94 °C for 30 s and 55 °C for 60 s, followed by 98 °C for 10 min, and 4 °C for 30 min. The cycled plate was then transferred and read in the FAM and HEX channels for ORF1 and N gene targets, respectively, using the QX200 reader (Bio-Rad).

The results were analysed using a droplet reader connected to a computer running the Quanta Soft Software (Bio-Rad). The feasibility of using TaqPath^{TM} COVID-19 RT-PCR mix for ddPCR was assessed by preliminary experiments on positive human samples opportunely diluted.

3.3.5. Identification of Coronaviruses by End-Point RT-PCR

To broaden the scope of our analysis, we also used three protocols to detect the general presence of coronaviruses by end-point RT-PCR. All assays were carried out with the AgPath-ID™ One-Step RT-PCR kit (Thermo Fisher Scientific, Waltham, MA, USA).

The first two protocols were carried out using the primers by Drosten et al. (2003) (Drosten et al., 2003) (see Table 2) with a thermal profile indicated by Amoroso et al. (2020) (Amoroso et al., 2020) or with a thermal profile indicated by Drosten et al. (2003) (Drosten et al., 2003) and modified as follows: 45 °C for 30 min; 95 °C for 10 min; 10 cycles of 95 °C for 10 s, 60 °C for 10 s (decreasing by 1 °C per cycle), 72 °C for 30 s; 40 cycles of 95 °C for 10 s, 56 °C for 10 s, and 72 °C for 20 s.

Table 2. Primers used for the detection of coronaviruses in the 240 bat (droppings or saliva) samples analysed. The amplification size is expressed as base pairs (bp). RdRp: RNA-dependent RNA polymerase.

Virus	Primer	Sequence	Amplification size (base pair)	Reference
Coronavirus	F	5’- GGGTTGGGACTATCCTAAGTGTGA -3’	251	(Drosten et al., 2003)
	R	5’- TAACACACAAACACCATCATC A -3’		
Coronavirus	F	5’-ACWCARHTVAAYYTNAARTAYGC-3’	452	(Vigen et al., 2008)
	R	5’- TCRCAYTTDGGRTARTCCCA -3’		

For both protocols, the reaction (final volume of 25 μL) contained 12.5 μL of AgPath-ID™ One-Step RT-PCR mix, 1 μL of enzyme mixture, and 1.25 μL of each of the two primers (10 μM).

In the third protocol, we used the primers mentioned by Vigen et al. (2008) (Vigen et al., 2008) and the reaction (final volume 25 μL) contained 12.5 μL of AgPath-ID™ One-Step RT-PCR mix, 1 μL of enzyme mixture, and 0.3 μL of each of two primers (10 μM). The thermal profile was modified in the denaturation step with a temperature of 95 °C (instead of 94 °C) and in the extension time using 30 s instead of 1 min.

All PCR products were analysed by an automated platform Tape Station 2200 (Agilent Technologies, Santa Clara, CA, USA), using the D1000 screentape system. In all the protocols carried out, we used the following positive controls: SARS-CoV-2 Delta variant, SARS-CoV-2 Omicron variant, canine coronavirus, bovine coronavirus, and feline coronavirus. Strains were all taken from field samples, cultivated on the appropriate cell substrate and nucleic acids were extracted before they were used in the PCR assays.

3.3.6. Sequencing

Bands of interest, (251 bp or 452 bp concerning the protocol used, see Table 2) were collected using the E-Gel system (Invitrogen™, Carlsbad, CA, USA) described by Gibson et al. (2010) (Gibson et al., 2010) and were either directly sequenced or underwent a second round of PCR using the same primers used in the first round.

Amplicons were sequenced as previously described (Amoroso et al., 2013). The nucleotide sequence similarity searches were performed using the Basic Local Alignment Search Tool (BLAST) server (http://www.ncbi.nlm.nih.gov/genbank/index.html) (accessed on 5 September 2021).

3.4. Results

We sampled 129 bats from three different provinces of the Lazio and Campania Regions (Figure 4). Bats were captured under the permission of the Italian Ministry of Ecological Transition based on the positive scientific assessment made by ISPRA prot. 22990, 5 May 2021. The collected bats belonged to 10 bat species (Table 3). Specifically, we took 127 nasal swabs and 113 faecal droppings for a total of 240 sample units. All samples, from all the three provinces investigated, gave negative results for SARS-CoV-2 when analysed with the RT-PCR TaqPath^{TM} COVID-19 RT PCR kit (Thermo Fisher Scientific, Waltham, MA, USA), which is the same kit used by Istituto Zooprofilattico Sperimentale del Mezzogiorno during the pandemic for the molecular assessment of COVID-19 positivity on human swab samples. These results were confirmed by also testing the samples with the droplet digital PCR (ddPCR), using the same kit. In the provinces in which the sampling was carried out, and especially in the province of Naples, there was a high circulation of SARS-CoV-2 from March 2020 (when the COVID-19 pandemic started) until July 2021, when sampling was completed. A total of 254,049 COVID-19 human cases were reported for the province of Naples out of 2,986,745 inhabitants (accounting for 8.5% of the population), 31,663 cases in the province of Frosinone (out of 472,559 inhabitants, i.e., 6.7% of the population) and 12,731 cases in the province of Benevento (out of 266,716 inhabitants, corresponding to 4.77% of the population). Data on the number of human COVID-19 cases were taken from https://lab24.ilsole24ore.com/coronavirus (accessed on 10 May 2022), and those on the number of province inhabitants (updated 1 January 2021) were taken from www.istat.it (accessed on 10 May 2022).

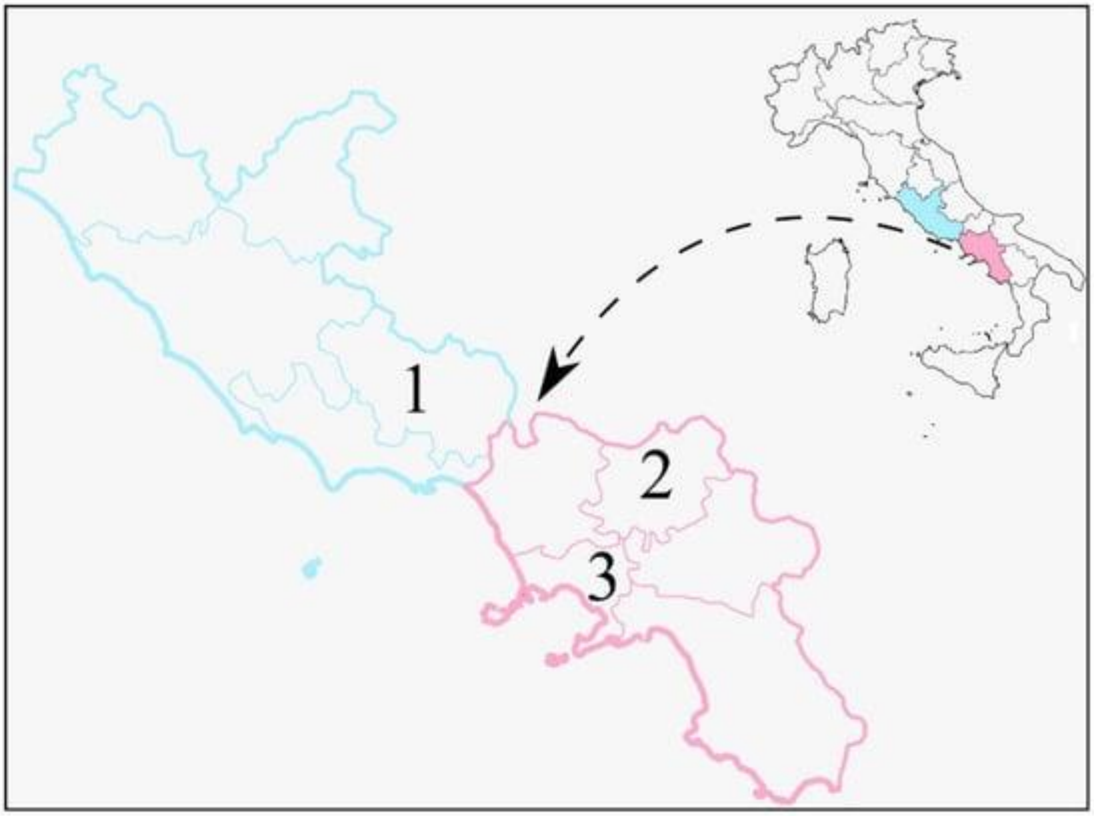

Figure 4. Regions of Central–Southern Italy where the bats were sampled. Sky blue colour = Lazio Region; Pink colour = Campania Region. Numbers identify the provinces where the sampling was carried out: 1—Frosinone; 2—Benevento; 3—Naples.

Table 3. The global conservation status of the bats examined according to the International Union for Conservation of Nature (IUCN) Red List and the location of the bat species involved in this study. The sex ratio is expressed as (males, females). VU = Vulnerable, EN = Endangered, NT = Near Threatened, NE = Not Evaluated, LC = Least Concern. Sites were categorised into urban and peri-urban.

Species	**IUCN Red list classification**	**Frosinone (Urban)**	**Benevento (Peri-urban)**	**Naples (Peri-urban)**
Miniopterus schreibersii	VU	(0,0)	(4,2)	(0,0)
Myotis capaccinii	EN	(0,0)	(2,1)	(0,0)
Myotis emarginatus	NT	(0,0)	(0,2)	(0,0)
Myotis crypticus	NE	(1,0)	(0,0)	(0,1)
Nyctalus leisleri	NT	(1,0)	(0,0)	(0,0)
Pipistrellus kuhlii	LC	(0,4)	(0,0)	(1,5)
Pipistrellus pipistrellus	LC	(0,1)	(0,0)	(0,0)
Hypsugo savii	LC	(0,4)	(0,0)	(13,76)
Rhinolophus ferrumequinum	VU	(0,0)	(1,0)	(0,0)
Rhinolophus euryale	VU	(0,0)	(3,7)	(0,0)

To search for other coronaviruses, an end-point RT-PCR was performed with three different protocols. All samples showed no specific bands except 12 samples that yielded faint amplicons close to the expected size. These amplicons were directly sequenced, providing very low-quality sequences that made them unusable. To improve amplicon yield, we carried out the second round of PCR. Again, sequencing revealed the very low quality of the sequences, most likely due to the non-specificity of the bands.

3.5. Discussion

In our study, we found no evidence of SARS-CoV-2 presence in 10 bat species (129 individuals) sampled in Central and Southern Italy. Sampling was concentrated in summer when most of the examined bat species congregate in nurseries in buildings, a kind of behaviour that might increase the risk of reverse zoonotic transmission. The bats we sampled included both species that typically roost in buildings, quite often shared with humans, such as *P. kuhlii*, *P. pipistrellus*, *H. savii*, and *R. ferrumequinum*, and species that roost in more natural locations, especially caves (*M. schreibersii*, *M. capaccinii*, *M. emarginatus*, *R. euryale*) or tree cavities (*N. leisleri*, *M. crypticus*). While some "cave-dwelling" species may also roost in buildings (*M. emarginatus*, *R. euryale*) in the study regions considered (D. Russo, pers. obs.), in all cases, sampling took place within ca. 2 km from the closest urban site, a distance easily covered by foraging bats (Altringham et al., 2011).

We also had negative results when analysing bats with generic PCR protocols, yet a band of the correct size was found for coronaviruses (CoVs) in 12 samples (corresponding to 9.8% of the total sample). However, none of the samples could be confirmed by sequencing and they were all considered negative. These data confirm those shown in a 2017 survey (Amoroso et al., 2018) that failed to find CoVs in 147 bats collected from three regions of Central–Southern Italy (Lazio, Campania, and Abruzzo) from 13 bat species (7 of which are featured in the present study). In (Amoroso et al., 2018), faint amplicons of the expected size in 10 bat samples could not be confirmed by sequencing. These data suggest that coronaviruses are absent in bat populations in the study area that we considered, but confirmation warrants further analysis.

Two non-mutually exclusive conditions may be hypothesized to explain our negative results. First, bats might have limited or no susceptibility to a productive infection of SARS-CoV-2, as suggested by laboratory work (Schlottau et al., 2020; Hall et al., 2021), despite the high similarity of this virus with certain bat coronaviruses (Temmam et al., 2022). While this may be the case, it is worth highlighting that over 1400 bat species exist worldwide and are found in virtually all regions of the globe except the polar ice caps, so any generalization based on small sample sizes, restricted geographic scope, and few species warrants caution. On the other hand, transmission from humans to bats may be unlikely because, in most cases, bats have little contact with humans. Even in urbanized areas, where bats roost in spaces of buildings that can be used by people, the chances that bats will find themselves at distances close enough to be reached by droplets breathed out by a positive person are slim.

Handling bats may be a prime way to infect them, so the "spillback" risk is higher when researchers or rehabilitators, both categories professionally handling bats, are involved. In Italy, rehabilitators and researchers were promptly informed of the potential spillback risk from the very beginning of the COVID-19 outbreak, so appropriate measures were taken following the guidelines provided by the IUCN (Medellin et al., 2020; Nunez et al., 2020) and the Agreement on the Conservation of Populations of European Bats (EUROBATS) (https://www.eurobats.org/node/2602) (accessed on 11 May 2020). This prompt reaction may have acted as significant mitigation of the reverse transmission risk. Prescriptions made by the IUCN relied on a Minimize, Assess, Protect (MAP) strategy. It is important to minimize or avoid bat handling when it is not necessary, and in case of necessity, bat handling should not take place when operators show any symptoms of COVID-19. Moreover, practices that reduce exposure must be adopted, such as wearing masks, and gloves, and always using disinfection (Medellin et al., 2020; Nunez et al., 2020). The EUROBATS panel of experts provided very similar recommendations (https://www.eurobats.org/node/2602) (accessed on 11 May 2020).

While contacts between bats and humans are rare and restricted to certain categories, the hypothesis that predation on bats by domestic cats might favour the transmission of SARS-CoV-2 from humans to bats cannot be ruled out (Salinas-Ramos et al., 2021). Domestic cats are well-known predators of over 45 bat species, and due to their frequent interactions with people, they might act as SARS-CoV-2 intermediate hosts between humans and bats (Salinas-Ramos et al., 2021). The recent spillover event of West Caucasian Bat Lyssavirus (WCBV) in a domestic cat from bats (supposedly, *Miniopterus schreibersii*) would provide further evidence of the role that domestic cats might play in facilitating epidemiological contact between humans and the wildlife that cats prey upon (Leopardi et al., 2021). Domestic animals, such as dogs and cats (28-Lauth et al., 2020) are susceptible to SARS-CoV-2, so we argue that the systematic surveillance of pets besides wildlife is highly important, especially where domestic animals are exposed to frequent encounters with wildlife.

We are aware that our work is preliminary, focusing on less than one-third of the bat species occurring in Italy and two out of twenty regions of the country, and that only a serological assessment of bat populations would prove the absence of infection. However, our study still provides first valuable picture, proving that bats did not shed the virus in a period of high viral circulation in humans. To our best knowledge, this is the first study dealing with the occurrence of SARS-CoV-2 in Italian bats, so we hope our contribution will encourage the establishment of systematic surveillance of wildlife and bats, to help prevent zoonoses as well as reverse zoonotic events that would put at risk human health, as well as endanger biodiversity conservation and management. Future work will include serological analyses at specific sites and extend surveillance to other species and regions to paint a more comprehensive picture of the situation. Moreover, the virus is evolving and new variants are systematically arising. Whether further mutations will make bats more susceptible to the virus has to be established and represents another good reason to systematically continue active surveillance.

We remark that active surveillance does not replace the careful prevention of the spillback processes, which requires the strict adoption of all precautions set by the IUCN, which aim to mitigate risks for the categories of people that have frequent contact with bats.

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M.G.A.; Writing— original draft, H.D., D.R. and M.G.A. All authors have read and agreed to the published version of the manuscript.

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4. Molecular Survey of *Toxoplasma gondii* in Wild Mammals of Southern Italy

Hiba Dakroub [1,2], Giovanni Sgroi [2], Nicola D'Alessio [2,3], Danilo Russo [1, *], Francesco Serra [2], Vincenzo Veneziano [3,4], Simona Rea [2], Alessia Pucciarelli [2], Maria Gabriella Lucibelli [2], Esterina De Carlo [2], Giovanna Fusco 2 and Maria Grazia Amoroso [2, *]

1 Laboratory of Animal Ecology and Conservation (AnEcoEvo), Department of Agriculture, University of Naples Federico II, 80055 Portici, Italy

2 Department of Animal Health, Istituto Zooprofilattico Sperimentale del Mezzogiorno, 80055 Portici, Italy

3 Osservatorio Faunistico Venatorio of Campania Region, Istituto Zooprofilattico Sperimentale del Mezzogiorno, 80055 Portici, Italy 4 Department of Veterinary Medicine, University of Naples Federico II, 80137 Naples, Italy

* Correspondence: danrusso@unina.it (D.R.); mamoroso@izsmportici.it (M.G.A.)

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4.1. Abstract

Systematic wildlife surveillance is important to aid the prevention of zoonotic infections that jeopardize human health and undermine biodiversity. *Toxoplasma gondii* is an opportunistic zoonotic protozoan that can infect all endothermic vertebrates, causing severe disease in immunocompromised humans and cases of congenital transmission. Humans can be infected by ingestion of raw meat containing bradyzoites or water contaminated by oocysts. In our study, we assessed the potential circulation of *Toxoplasma gondii* in wild mammals by performing surveillance in the Campania region (southern Italy) and surveyed its presence from 2020 to 2022 within the framework of the Regional Plans for Wildlife Surveillance. In detail, 211 individuals belonging to five wild mammals (wolf, fox, wild boar, badger, and roe deer) underwent necropsy and the organs were analyzed by real-time PCR for the detection of the parasite. *Toxoplasma gondii* was found in 21.8% (46/211) of the subjects examined. No statistically significant differences were noticed between the prevalence and the host's trophic level or age, rejecting the hypotheses that *Toxoplasma gondii* will have a higher prevalence in top predators and adult individuals, respectively. Our work emphasized the high circulation of *Toxoplasma gondii* in wildlife and remarked on the critical role of anthropized areas where domestic cats and wildlife may come into contact, urging a systematic surveillance.

Keywords: parasite; prevalence; apicomplexan; *Canis lupus*; *Meles meles*; *Capreolus capreolus*; *Sus scrofa*; *Vulpes vulpes*; zoonosis

4.2. Introduction

Toxoplasma gondii (Conoidasida, Sarcocystidae) is an apicomplexan protozoan that causes a zoonotic infection known as toxoplasmosis. This parasite is one of the most resilient and persistent living parasites, able to infect many endothermic vertebrates including humans (Bokaba et al., 2022). The Food and Agriculture Organization of the United Nations (FAO) identified toxoplasmosis among the 10 most important foodborne diseases (Kobayashi et al., 2021). The definitive hosts of *Toxoplasma gondii* are wild and domestic felids, since these animals facilitate the parasite's sexual recombination and shed millions of stable unsporulated oocysts into the environment through their faeces (Augusto et al., 2021). Yet, the intermediate host range of *Toxoplasma gondii* is incredibly broad, including humans, domestic animals, and wild vertebrates (Stelzer et al., 2019). These intermediate hosts support the asexual forms of tachyzoite and bradyzoite tissue cysts of the parasite, which invade the host's small intestine after consumption (Augusto et al., 2021). Humans can be infected via different ways: (1) by eating undercooked meat of animals harbouring bradyzoites in the tissue cysts; (2) by ingesting food or water contaminated with oocysts shed with faeces; (3) by blood transfusion or organ transplantation; and (4) through the placenta, from mother to the foetus with tachyzoites (Dubey at al., 2008; Elmore at al., 2010). During the initial infection phase of an intermediate host, comprising humans, *Toxoplasma gondii* replicates rapidly and spreads throughout the tissues, including the brain (acute toxoplasmosis). Eventually, parasite replication slows down, and the protozoa cluster together in tissue cysts (latent toxoplasmosis). Humans with latent toxoplasmosis who become immunocompromised may develop reactivated toxoplasmosis, in which the dormant parasites in the tissue cysts will start replicating again. This reactivation can cause severe flu-like symptoms, blurred vision, or toxoplasmic encephalitis (Reiling et al., 2019).

Recently, many epidemiological studies associated latent toxoplasmosis with a wide variety of cognitive and neuropsychiatric disorders including Alzheimer's, bipolar disorders, epilepsy, obsessive compulsive disorders, and schizophrenia (Milne at al., 2020). This association seems to be determined by the interference between the parasite and the expression of many neurotransmitters (Milne at al., 2020). Despite this new and important evidence and considering that the parasite is estimated to persist chronically in about 30% of the human population, toxoplasmosis is underestimated in terms of monitoring, preventing, and treating it (Dubey at al., 2020). Studies that contribute to a better understanding of the pathogen's distribution and prevalence, as well as the factors that influence its prevalence, are, therefore, of special importance. Thus, the present study provides information that could be used in future studies that aim to estimate the prevalence of *Toxoplasma gondii* in wild mammals from southern Italy (Campania region) to better understand the parasite's life cycle, its transmission dynamics, and the risk to public health (Gazzonis et al., 2021), and to provide useful information for wildlife management and public health protection. We selected the Campania region due to its high human density and the proximity between humans and wildlife, exacerbated by many free-ranging domestic cats, which highly increases the risk of zoonotic transmission of this pathogen, as well as others (e.g., (Salinas-Ramos et al., 2021)). In general, the host's trophic level may influence exposure risk since top predators of endothermic vertebrates will be exposed to increased infection risks through the tissue-cyst transmission route (Wilson et al., 2020). In our study, we covered all mammal trophic levels from top predators to prey through mesocarnivores. Specifically, we tested the hypothesis that species-specific ecological traits related to trophic levels will lead to different exposure rates to *Toxoplasma gondii* through oocysts and tissue cysts. We expected a higher prevalence in top predators, intermediate in mesocarnivores, and lowest in mammal prey. While it is believed that sex does not affect the prevalence of the parasite (Esteves et al., 2014), age may affect the presence of *Toxoplasma gondii* in wild animals, as older animals are more likely to be infected (De sousa et al., 2014; Formenti et al., 2015; Gering et al., 2021). Therefore, we also hypothesized that the prevalence of *Toxoplasma gondii* will change according to age, suggesting that the parasitic prevalence will be higher in adults, intermediate in sub-adults, and lower in juveniles. Lastly, the infection rate could be also related to the different geographic sampling areas. In southern Italy, oocysts are expected to be more common in peri-urban areas where free-ranging domestic cats (Felis catus), both stray cats and pets, are allowed outdoors, acting as the most relevant definitive hosts of *Toxoplasma gondii* (Shapiro et al., 2019). Most domestic cats found in urban areas are mainly kept indoors, apart from the limited free-ranging cat colonies protected by Italian national legislation (Natoli et al., 2006). However, free-ranging cats are widespread in rural and especially peri-urban areas of southern Italy, setting the scene for more frequent contact with wild mammals. In this context, we, therefore, hypothesized that wild mammals will encounter oocysts more frequently in areas where cats are freely moving, i.e., peri-urban sites, where a higher rate of *Toxoplasma gondii* is expected.

4.3. Materials and Methods

4.3.1. Study Area and Sample Collection

We sampled five mammal species between January 2020 and November 2022, from different areas of the Campania region (southern Italy). The region is mainly hilly and extends from 0 to 1890 m above sea level. The climate is Mediterranean with dry summers and rainy winters (e.g., (Fusco et al., 2007)). To test our hypotheses, we considered a total of 211 individuals as follows: wolves (*Canis lupus* n = 14), foxes (*Vulpes vulpes* n = 71), badgers (*Meles meles* n = 22), and roe deer (*Capreolus capreolus* n = 14), all found dead, while wild boars (*Sus scrofa* n = 90) were either found dead or killed by hunters. Additionally, we analysed stone martens (*Martes martes* n = 3), porcupines (*Hystrix cristata* n = 4), and otters (*Lutra lutra* n = 5), whose sample sizes were too small for statistical analysis. Since all procedures followed the Italian and EU legislation, as part of the Regional Plans for Wildlife Surveillance (Louhimies et al., 2002), no approval from the ethical committees was needed. All individuals underwent a necropsy on the premises of the Istituto Zooprofilattico Sperimentale del Mezzogiorno (Portici, southern Italy), by professional staff (veterinarians and laboratory technicians) in a necropsy room. The organs that were collected for subsequent analysis depended on the animal’s condition. In general, if possible, brains, hearts, and/or muscles were removed with sterile scalpels, dissected, split out in sterile tubes, delivered within 24 h to the laboratory for biotechnological investigation and stored at −20 ◦C before DNA extraction.

4.3.2. Nucleic Acid Extraction

Twenty-five mg of each organ were minced with a sterile blade and transferred to a sterile Eppendorf containing 1 mL of pyrophosphate-buffered saline (PBS) solution. Samples were homogenized by TissueLyser (Qiagen) with one stainless steel bead for 3 min and centrifuged at 13,000 rpm for 3 min.

Nucleic acids were extracted from 200 µL of the homogenized samples using the MagMax^{TM} Viral/Pathogen II Nucleic Acid Isolation Kit (Applied Biosystems, Waltham, MA, USA), following the manufacturer's instructions. Extracted nucleic acids were eluted in 80 µL elution buffer and immediately analysed by real-time PCR or stored at −20 ◦C until further processing. PCR inhibitors likely present in the samples were monitored by adding an external process control (EPC), namely murine norovirus (Tofani et al., 2022), 5 µL of which (107 PFUmL−1) was spiked in each sample prior to extraction. EPC was amplified separately before testing for *Toxoplasma gondii* in each sample by real-time PCR with the following primers: MNoV F 50 -CACGCCACCGATCTGTTCTG-30 and 50 -GCGCTGCGCCATCACTC-30; and probe FAM-CGCTTTGGAACAATG-MGB-NFQ with the thermal profile indicated in the literature (Baaert et al., 2008). Results were analysed as already described (Amoroso et al., 2021).

4.3.3. Molecular Analysis

A real-time PCR was used to detect a small part of the *Toxoplasma gondii* B1 gene, as described by Sgroi et al. (2020). Briefly, 5 µL of template DNA was added to a reaction mixture in a final reaction volume of 25 µL containing PCR universal mastermix 1X, 0.5 µM of each primer (forward primer TOXO-F' 50 -TCCCCTCTGCTGGCGAAAAG0 -30 and reverse primer TOXO-R' 50 -AGCGTTCGTGGTCAACTATCGATT0 -30) and 0.2 µM of TaqMan probe (2 µM, 6FAM-TCTGTGCAACTTTGGTGTATTCGCAG-TAMRA) (Sgroi et al., 2020). The thermal profile included an initial activation at 95 ◦C for 15 min, followed by 45 PCR cycles of 95 ◦C for 15 s and 60 ◦C for 1 min. Nuclease-free water was included as a negative control and genomic DNA from *Toxoplasma gondii* was obtained from the America Type Culture Collection (ATCC 50174D LGC Standards Italy) used as reference control. The amplifications were performed on a QuantStudio 5 real-time PCR system (Applied Biosystems, Foster City, CA, USA) thermal cycler.

4.3.4. Data Classification

To investigate possible spatial variation in *Toxoplasma gondii* prevalence among wild mammals, the analysed subjects were categorized according to the species' trophic level as top predators (wolves), mesopredators (foxes and badgers), or prey (wild boars and roe deer). Additionally, we classified them by sex (females or males) and by age: juveniles Pathogens 2023, 12, 471 4 of 10 (2 years) (Buesching et al., 2002; Bier et al., 2020; Skonhoft et al., 2021). Age assessment was carried out empirically by comparing the biometric values of each individual with those known for the different species. The areas in which we collected animals were divided into three geographic zones: urban, peri-urban, or rural. Urban areas were characterised by human settlements with a high density of infrastructures and built environments, while areas considered rural occurred outside towns and cities, were dominated by farmland, and had very low occurrence or complete absence of buildings. We classified peri-urban areas following the UNESCO classification, according to which peri-urban areas are zones of transition from rural to urban land uses located between the outer limits of urban and rural environments (https://en.unesco.org/events/peri-urban-landscapes-water-foodand-environmental-security, accessed on 10 October 2022). The spatial distribution of prevalences was investigated by building a map with locations of sampling obtained via ArcGIS (version 10.3; ESRI, Redlands, CA, USA).

4.3.5. Statistical Analysis

Only wolves, foxes, badgers, wild boars, and roe deer had sufficiently large (>10) sample sizes for statistical analysis. The remaining species were not included in the analysis due to insufficient sample size, and prevalence data for them are provided in Supplementary Tables S1 and S2. Contingency tables were analysed to assess the differences in prevalence associated with age, sex, sampling area, and organ infection prevalence. A p-value < 0.05 was considered statistically significant. The Fisher's exact test was used and confidence intervals (CI) at 95% were also calculated. All statistical analyses were carried out with the jamovi software tool (http://www.jamovi.org/, accessed on 10 October 2022).

4.4. Results

A total prevalence of 21.8% (n = 46 positive samples out of 211) for *Toxoplasma gondii* was obtained from molecular analysis of the samples collected. According to species, the prevalence was as follows: wolf (n = 4/14; 28.6%, CI: 11.7–54.6), badger (n = 6/22; 27.3%, CI: 13.1–48.1), fox (n = 17/71; 23.9%, CI: 15.8–36.3), wild boar (n = 17/90; 18.9%, CI: 12.1–28.2), and roe deer (n = 2/14; 14.3%, CI: 4.0–39.9). No statistically significant difference in prevalence was found among species (Table 4).

Table 4. Significance levels of Fisher's exact test in pairwise comparisons of *Toxoplasma gondii* prevalence between mammal species and trophic levels.

Species	*P*
Wolf vs fox	0.740
Wolf vs wild boar	0.474
Wolf vs roe deer	0.648
Wolf vs badger	1.000
Fox vs wild boar	0.435
Fox vs roe deer	0.726
Badger vs fox	0.752
Wild boar vs roe deer	1.000
Badger vs wild boar	0.383
Badger vs roe deer	0.441
Top predators vs mesopredators	0.748
Top predators vs mammal prey	0.470
Mesopredator vs mammal prey	0.269

From a visual assessment of data distribution, we detected a decreasing trend (Figure 5) from apex predators (n = 4/14; 28.6%, CI: 11.7–54.6) to mesopredators (n = 23/93; 24.7%, CI: 17.1–

34.4), and prey mammals (n = 19/104; 18.2%, CI: 12.0–26.8), but the pairwise comparisons of the prevalence between the groups was not significant (Table 4).

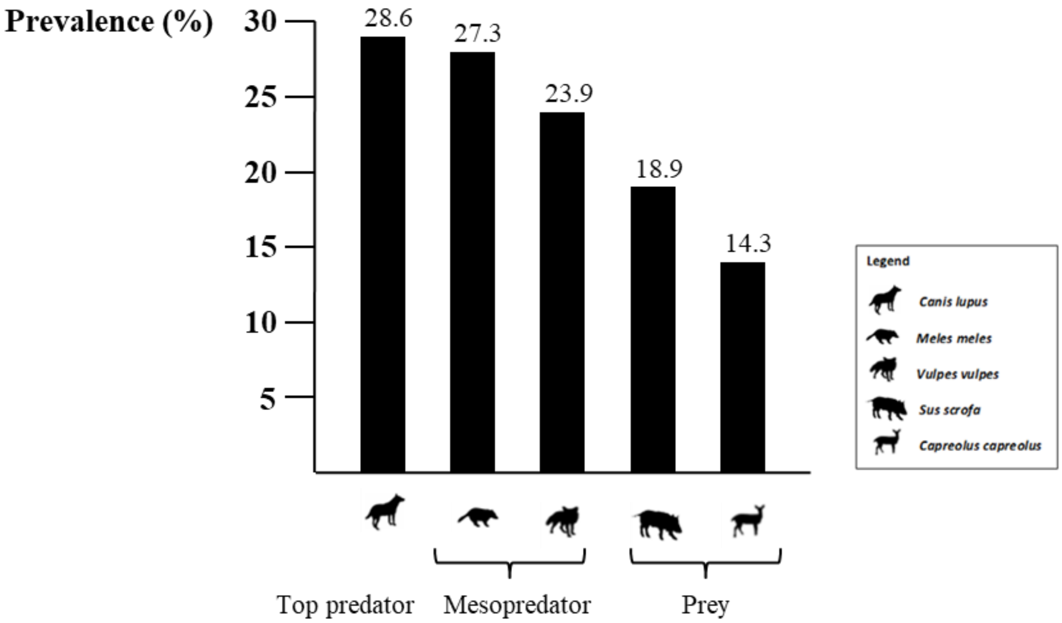

Figure 5. Molecular prevalence of *Toxoplasma gondii* in different synanthropic wildlife species of southern Italy, 2020–2022.

We found individuals positive to *Toxoplasma gondii* in all provinces of the Campania region (Figure 6), with no statistical difference between provinces (χ2 = 0.73; p = 0.13).

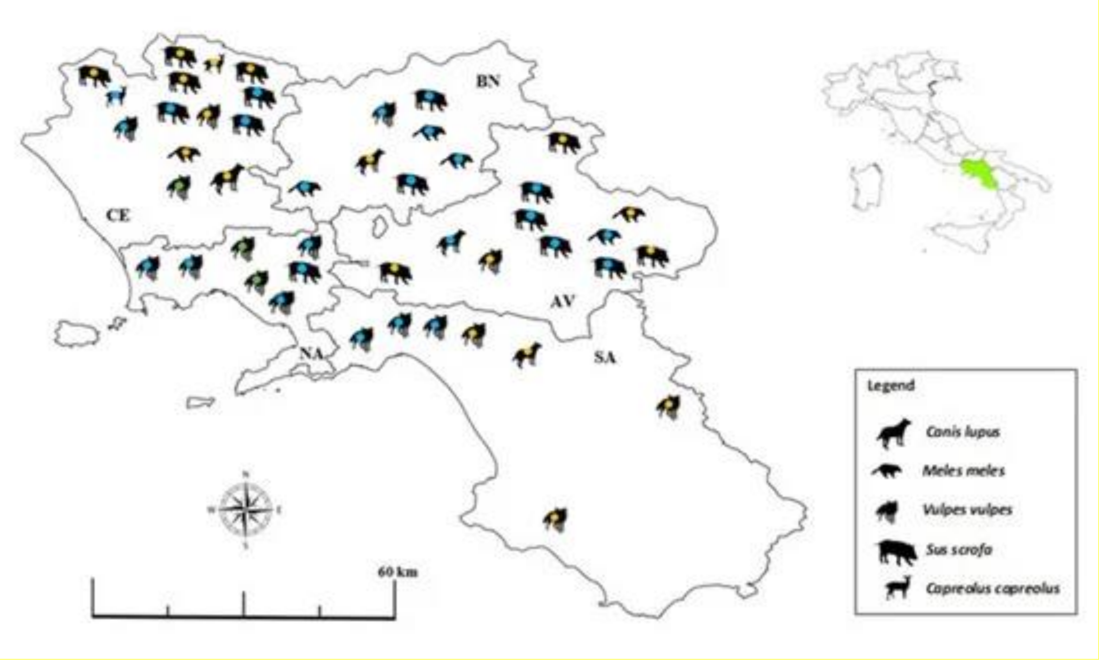

Figure 6. Map showing the distribution of synanthropic mammals (n = 46) that tested positive for *Toxoplasma gondii* DNA in different provinces (i.e., AV, Avellino, BN, Benevento, CE, Caserta, NA, Napoli, SA, Salerno) of the Campania region, southern Italy, in 2022022. Green = urban area; blue = peri-urban area; yellow = rural area.

Age and sex did not influence positivity in any of the species analysed. Only a boarder significance in the sex was associated with prevalence in the wild boars (p = 0.053), where positivity was more frequently associated with males (Table 5).

For wolves, land use comparisons were only possible between peri-urban and rural areas because no individual was found in urban areas (Table 5). Although the sample size was small, wolves from rural areas were more frequently positive than those from peri-urban areas. Instead, wild boars found in peri-urban areas were more frequently positive than those from rural and urban areas, respectively (Table 5).

Out of the 465 organs analysed, the highest prevalence rate was found in the heart (25/163, 15.3%), followed by the brain (12/112, 10.7%) and the muscle (18/190, 9.2%), but such differences were not statistically significant (Table 6).

Table 5. Prevalence of *Toxoplasma gondii* DNA in synanthropic mammal species of southern Italy according to sex, age, and dominant land use in 2020–2022. Missing categories (indicated with "-") refer to the absence of samples.

Variable	Species				
	Wolf	Fox	Wild boar	Roe deer	Badger
	Pos/Tot (%)	Pos/Tot (%)	Pos/Tot (%)	Pos/Tot (%)	Pos/Tot (%)
Sex					
Male	2/5 (40.0)	9/39 (23.1)	11/37 (29.7)	2/12 (16.7)	5/15 (33.3)
Female	2/9 (22.2)	8/32 (25.0)	6/53 (11.3)	0/2 (0)	1/7 (14.3)
	p = 0.580	*p* = 1.000	*p* = 0.053	*p* = 1.000	*p* = 0.610
Age					
Juvenile	-	3/15 (20.0)	1/10 (10.0)	-	1/2 (50.0)
Sub-adult	0/2 (0)	3/15 (20.0)	10/52 (19.2)	0/5 (0)	1/5 (20.0)
Adult	4/12 (33.3)	11/41 (26.8)	6/28 (21.4)	2/9 (22.2)	4/15 (26.7)
	p = 1.000	*p* = 0.866	*p* = 0.858	*p* = 1.000	*p* = 0.799
Dominant land use					
Urban	-	3/16 (18.7)	0/7 (-)	-	0/1 (-)
Peri-urban	1/10 (10)	9/40 (22.5)	10/26 (38.5)	1/10 (10.0)	4/15 (26.7)
Rural	3/4 (75.0)	5/15 (33.3)	7/57 (12.3)	1/4 (25.0)	2/6 (33.3)
	p = 0.040	*p* = 0.695	*p* = 0.010	*p* = 0.505	*p* = 1.000

Table 6. Prevalence of *Toxoplasma gondii* DNA in synanthropic mammal species of southern Italy in 2020-2022 according to the organs examined.

Species	*P* value	Organs		
		Heart	Brain	Muscle
		Pos/Tot (%)	Pos/Tot (%)	Pos/Tot (%)
Wolf	*p* = 0.822	3/11 (27.3)	1/11 (9.1)	1/7 (14.3)
Fox	*p* = 0.485	8/48 (16.7)	4/48 (8.3)	6/61 (9.8)
Wild boar	*p* = 0.580	10/82 (12.2)	5/37 (13.5)	7/86 (8.1)
Roe deer	*p* = 0.405	1/4 (25.0)	Not performed	1/14 (7.1)
Badger	*p* = 0.1000	3/18 (16.7)	2/16 (12.5)	3/22 (13.6)
Total	*p* = 0.228	25/163 (15.3)	12/112 (10.7)	18/190 (9.5)

4.5. Discussion

Our survey revealed a high prevalence of *Toxoplasma gondii* (21.8%) in wild mammals found in different territories of the Campania region (southern Italy), i.e., 1 out of 4.4 synanthropic wild mammals harboured the parasite DNA. This finding confirmed the pattern known from other European regions. For instance, Calero-Bernal et al. (2015) found an overall prevalence of T. gondii infection of 32.2% in 183 wild mammals from southwestern Spain belonging to six different species, three of which (foxes, wild boars, and roe deer) were in common with the present study (Calero-Bernal et al., 2015). We also found that although mammals at higher trophic levels exhibited a higher prevalence of *Toxoplasma gondii*, no statistically significant differences were noticed between the categories. Ferroglio et al. (2014) (Ferroglio et al., 2014) showed a higher prevalence in carnivorous and omnivorous wild mammals (red fox and wild boar) than in herbivores (red deer and chamois), which was interpreted because of the cumulative effect of the parasite's predator– prey cycle (see also (Smith et al., 1995)). This would link to the higher probability of a carnivorous or omnivorous species ingesting infected tissues vs. that of a herbivore, which may only ingest oocyst-contaminated food plants, soil, and/or drinking water (Wilson et al., 2020). The different result herein obtained was probably associated with the high degree of anthropization typical of the Campania region, and the increasingly synanthropic habits of the mammal species we considered (Sgroi et al., 2020; Ossi et al., 2020; Castillo-Conreras et al., 2021; Torretta et al., 2022). All factors increased the likelihood of pathogen-spread in areas where free-ranging domestic cats were abundant and their direct or indirect contacts with wildlife frequent. Additionally, all species considered in this study, except the roe deer, were carnivorous or omnivorous (Pigozzi et al., 1991; Meriggi et al., 2011; Ballari et al., 2014), which may have partially masked the predicted pattern, weakening its statistical significance (while the visual trend was retained). The high circulation of the parasite among syntropic mammal species we found, besides being characterized by the above-mentioned lack of differences across trophic levels, was also confirmed by the absence of statistically significant differences in overall prevalence among provinces of the study region. Like previous work (e.g., (Esteves et al., 2014; Herrero et al., 2016)), we found no difference among animal sex except a boarder significance (p = 0.053) in the wild boars, with males more often positive than females. A possible explanation for this is that males tend to more frequently be in contact with potential sources of infection, since they typically show larger home ranges than females (Laguna et al., 2021). Moreover, wild boar males are typically solitary and more risk-prone than females (living in groups along with their young), and so, males may tend to use food-rich, anthropized habitats more than females (Said et al., 2012); all the more in areas such as the one we considered, where wild boar is routinely hunted. In addition, solitary males can more easily feed on the carcasses of animals injured in hunting (e.g., wild boars and foxes) by assuming cysts with bradyzoites and/or by carrying out a scavenging action (Said et al., 2012). In human-altered habitats, contact with cat-driven sources of infection is much more likely, which would explain the sex-biased prevalence we recorded (Castillo-Contreras et al., 2021). Wild boars were recently found to be attracted by cat food in anthropized areas, a concerning situation in terms of domestic cat-to-wild boar transmission of pathogens, especially *Toxoplasma gondii* (Castillo-Contreras et al., 2021), and solitary individuals such as males were more likely to obtain access to this kind of food source without being noticed and driven out. Unlike previous work (Esteves et al., 2014), we found no difference among age classes in the rates of positivity likely due to the limited sample sizes we considered, but also to the high circulation of the parasite in the environment, nullifying age-biased patterns. In general, older animals were more likely to have exposure with the parasite than younger ones, having a higher probability of being infected (Esteves et al., 2014), but this general pattern is likely to change considerably under different environmental and epidemiological conditions. Land use showed limited influence over positivity across the species considered, probably as a result of the high circulation of free-ranging cats, and as a consequence of the protozoan, in the study area. A borderline significance was observed in wolves, suggesting that in rural areas, these would be more affected than in peri-urban environments. Wolves in rural environments may have high probability to be in contact with Toxoplasma-infected prey, such as wild boars, the wolf's main prey in the Italian Apennines (e.g., (Mori et al., 2017)). However, this result must be taken cautiously due to the very small sample size available and the typically large home ranges shown by resident wolves, spanning over territories well over 100 km2 across many habitat types, or even more dispersing individuals, moving over up to thousands of km (Morales-Gonzalez et al., 2022). Classifying such highly opportunistic and mobile predators according to the land use of the area where they died may have little ecological meaning. The other species that showed a land use-biased positivity, the wild boar, had a clearer pattern, with subjects dwelling in peri-urban areas more frequently positive than those in urban or rural sites. This was probably explained in terms of the higher likelihood of contact with free-ranging cats typical of such environments, as discussed above. We also found, as in previous studies (Sakban et al., 2020), that the tropism of infection herein observed was equal for all organs considered. This record is in agreement with previous surveys by Alves et al. (2019), who observed the viability of *Toxoplasma gondii* cysts in organs of pigs experimentally infected with 3 × 103 oocysts of a *Toxoplasma gondii* isolate, demonstrating that the parasite can form tissue cysts in all organs (Alves et al., 2019). This study revealed a high circulation of *Toxoplasma gondii* in wild mammals of southern Italy, which may represent public health concern due to the virulence of the parasite in humans. The prevalence found in wild boar samples, especially muscles, highlights the relevance of these findings for public health since this species is commonly used to prepare traditional raw meat products, potentially representing an important source of *Toxoplasma gondii* infection to consumers. Wild boar is likely to play a major role in human infection as well as in the epidemiological cycle of *Toxoplasma gondii* infection (Rostami et al., 2017). The consumption of raw meat products from wild boar muscles also brings about a risk of other zoonotic pathogen infections (De Sabato et al., 2020). Once more, these results highlight the urgent need to implement surveillance of zoonotic pathogens in wildlife, especially those related to human consumption. The incidence of acute human toxoplasmosis hospitalizations in the Campania region is of 0.72/per 100,000 inhabitants, as reported by hospital discharge records (Pepe et al., 2021). Most patients were aged less than 1 year of age, followed by adults between 25 and 44 years of age with no gender difference (Pepe et al., 2021). Health problems related to toxoplasmosis are, however, highly underestimated, as the parasite infection is linked to many chronic diseases (Torrey et al., 2003; Xiao et al., 2018). Free-roaming domestic cats play an important role in spreading toxoplasmosis with marked impacts on biodiversity and human health. A science-based approach for better management of free-roaming cats is required from a broader political and legislative perspective, regulated by the consensus in the animal ecology, conservation, and welfare communities (Lepczyk et al., 2022). In Italy, urban free-roaming cats living in colonies are protected by the law (no. 281/1991), namely: (i) cats have the recognized right to live free; (ii) neutering of cats by the Veterinary Services of the Local Health Unit is compulsory; (iii) cat caretakers are institutionalized (Natoli et al., 2006). Although this degree of protection has certainly improved the conditions of stray cats, its outcome in terms of consequences for urban animal biodiversity as well as human health is still potentially concerning. We propose these colonies be systematically monitored for their effects on wildlife and the possible zoonotic consequences assessed to develop appropriate management strategies (Salinas-Ramos et al., 2021). Our findings highlighted the need to establish surveillance programs and preventive strategies in a multidisciplinary one health approach to the monitoring of wildlife species, to protect biodiversity and mitigate the risk of zoonotic transmission to humans. Accordingly, more efforts by the health stakeholders are required for the game meat inspection, such as for wild boar and roe deer foodstuffs, in order to prevent *Toxoplasma gondii* infections to consumers.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pathogens12030471/s1, Table S1: Prevalence of *Toxoplasma gondii* DNA in synanthropic wildlife species of southern Italy according to sex, age, and area, 2020–2022. Missing categories (indicated with "-") refer to the absence of samples; Table S2: Prevalence of *Toxoplasma gondii* DNA in synanthropic wildlife species of southern Italy according to the organs examined, 2020–2022. Missing categories (indicated with "-") refer to the absence of samples.

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5. Conclusions

The investigation into zoonoses and reverse zoonoses in wildlife populations, approached through the lens of the One Health paradigm, has illuminated critical aspects of infectious disease dynamics within European wildlife (Mubareka et al., 2023). The exploration of coronaviruses in European bats, particularly the assessment of SARS-CoV-2 circulation, and the molecular survey of *Toxoplasma gondii* in wild mammals of Southern Italy, has contributed substantial insights into the complex interactions between wildlife, pathogens, and human health.

In Chapter 1, the examination of coronaviruses within European bat populations underscores the significance of understanding viral prevalence and diversity among wildlife reservoirs (Wong et al., 2007). The detection and characterization of these viruses not only expanded our knowledge of their presence in bat species but also emphasized the necessity of continued surveillance and monitoring to mitigate potential spillover events (Phelps et al., 2019). In addition, thos monitoring studies indicated no evidence for circulation of SARS-CoV-2 related viruses in European bat populations before starting the Covid-19 pandemic. This chapter highlights the importance of proactive measures in understanding and managing emerging infectious diseases.

Chapter 2 provides a critical assessment of SARS-CoV-2 circulation in bats within Central–Southern Italy. The findings shed light on the potential reservoirs and transmission dynamics of this novel coronavirus in wildlife populations. This study serves as an essential baseline for ongoing surveillance efforts, informing public health strategies aimed at preventing future zoonotic outbreaks and promoting early detection and response mechanisms. Despite heightened concerns about zoonotic transmission and the role of bats in harboring coronaviruses, our rigorous study, conducted with meticulous sampling and robust methodologies, reveals an absence of SARS-CoV-2 among the examined bat species.

The absence of SARS-CoV-2 in the sampled bat populations challenges the prevailing assumptions regarding the direct involvement of bats as natural hosts or reservoirs for this specific coronavirus strain in the investigated region. This finding is significant in guiding our understanding of the transmission dynamics and potential reservoirs of SARS-CoV-2, prompting a reevaluation of the presumed role of bats in the epidemiology of this virus within this specific geographic area.

While our study did not identify SARS-CoV-2 in the examined bat populations, the absence of evidence in this case does not negate the importance of continued surveillance and monitoring efforts. The complex nature of zoonotic transmissions necessitates ongoing vigilance and comprehensive surveillance strategies to detect potential reservoirs and track the evolution of the virus within wildlife populations (Vandegrift et al., 2011). The findings underscores the need for broader investigations across various species and geographic regions to comprehensively comprehend the role of wildlife in the transmission and maintenance of SARS-CoV-2. Continued research and surveillance efforts are imperative to comprehensively ascertain the role of wildlife in the transmission and potential spillover of SARS-CoV-2, ultimately contributing to effective public health strategies and a more profound understanding of zoonotic diseases (Olival et al., 2020).

The investigation detailed in Chapter 3 concerning the molecular survey of *Toxoplasma gondii* in wild mammals of Southern Italy reveals the presence and prevalence of this protozoan parasite among diverse mammalian species. This underscores the intricate web of interactions between wildlife, domestic animals, and humans, highlighting the need for comprehensive strategies to manage and mitigate the risks associated with zoonotic transmission (Johnson et al., 2021).

Collectively, these studies emphasize the necessity of a multidisciplinary One Health approach in comprehensively addressing zoonoses and reverse zoonoses. They underscore the interconnectedness of human, animal, and environmental health, necessitating collaborative efforts among various disciplines to effectively monitor, prevent, and manage the transmission of infectious diseases at the wildlife–human interface (Sharan et al., 2023).

Preventing zoonoses and pandemics requires a comprehensive and interconnected approach that goes beyond simple reliance on surveillance measures (Gomes et al., 2022). The undeniable link between environmental crises and the emergence of infectious diseases underscores the urgency of addressing root causes (Logan et al., 2021). Deforestation, encroachment of urbanization into natural habitats, and the consumption of bushmeat collectively form open gates for new zoonoses, exemplified by the likes of *Toxoplasma* and coronaviruses (Vicente et al., 2021). While surveillance remains vital, it is but one piece of the puzzle. Embracing sustainable forestry practices, harmonizing urban development with conservation, and promoting responsible wildlife trade regulations are equally essential (Rodriguez Franco et al., 2023). By recognizing the intricate relationship between human activities and the health of our ecosystems, we can fortify our defenses against potential pandemics, ensuring a healthier and more harmonious coexistence between humanity and the natural world (Munasinghe et al., 2020). The insights garnered from these investigations provide valuable contributions to our understanding of zoonotic disease dynamics. They advocate for the continued integration of One Health principles in research, policy-making, and public health interventions to safeguard both human and wildlife populations from the threats posed by emerging infectious diseases.

In conclusion, this doctoral thesis delves deep into the intricate dynamics of zoonoses and reverse zoonoses within wildlife populations, employing a comprehensive and integrative One Health approach. The central focus of our investigation focuses on coronaviruses in bats and *Toxoplasma gondii* in wildlife, serving as enlightening case studies that underscore the complexity of the relationships between human, animal, and environmental health.

References

1. Alanagreh, L., Alzoughool, F., & Atoum, M. (2020). The human coronavirus disease covid-19: Its origin, characteristics, and insights into potential drugs and its mechanisms. *Pathogens*, 9(5). https://doi.org/10.3390/pathogens9050331
2. Alexander, K. A., Lewis, B. L., Marathe, M., Eubank, S., & Blackburn, J. K. Modeling of wildlife-associated zoonoses: applications and caveats. *Vector-borne and zoonotic diseases*, 2012, 12(12), 1005-1018. https://doi.org/10.1089/vbz.2012.0987
3. Alluwaimi, A. M., Alshubaith, I. H., Al-Ali, A. M., & Abohelaika, S. The Coronaviruses of Animals and Birds: Their Zoonosis, Vaccines, and Models for SARS-CoV and SARS-CoV2. *Frontiers in Veterinary Science*, 2020, 7(9), 1–12. https://doi.org/10.3389/fvets.2020.582287
4. AL-QAHTANI, Ahmed A. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): emergence, history, basic and clinical aspects. *Saudi Journal of Biological Sciences*, 2020, 27(10), 2531-2538. https://doi.org/10.1016/j.sjbs.2020.04.033
5. Altringham, J. D. Bats: from evolution to conservation, 2nd edition; Oxford University Press,2011, 324.
6. Alves, B.F.; Oliveira, S.; Soares, H.S.; Pena, H.F.J.; Conte-Junior, C.A.; Gennari, S.M. Isolation of Viable Toxoplasma Gondii from Organs and Brazilian Commercial Meat Cuts of Experimentally Infected Pigs. *Parasitology Research,* 2019, 118, 1331–1335. https://doi.org/10.1007/s00436-019-06229-6
7. Amoroso, M. G.; Corrado, F.; De Carlo, E.;Lucibelli, M. G.;Martucciello, A.; Guarino, A.;&Galiero, G. Bubaline herpesvirus 1 associated with abortion in a Mediterranean water buffalo. *Research in Veterinary Science*, 2013, 94(3), 813-816. https://doi.org/10.1016/j.rvsc.2012.12.009
8. Amoroso, M. G.; Russo, D.; Lanave, G.; Cistrone, L.; Pratelli, A.; Martella, V.; ... & Fusco, G. Detection and phylogenetic characterization of astroviruses in insectivorous bats from Central-Southern Italy. *Zoonoses and public health*, 2018, 65(6), 702-710. https://doi.org/10.1111/zph.12484
9. Amoroso, M. G.;Lucifora, G.; Degli Uberti, B.; Serra, F.; De Luca, G.; Borriello, G.; ... & Fusco, G. Fatal interstitial pneumonia associated with bovine coronavirus in cows from southern Italy. *Viruses*, 2020, 12(11), 1331. https://doi.org/10.3390/v12111331

10. Amoroso, M.G.; Serra, F.; Esposito, C.; D'Alessio, N.; Ferrara, G.; Cioffi, B.; Anzalone, A.; Pagnini, U.; de Carlo, E.; Fusco, G.; et al. Prevalence of Infection with Porcine Circovirus Types 2 and 3 in the Wild Boar Population in the Campania Region (Southern Italy). *Animals*, 2021, 11, 3215. https://doi.org/10.3390/ani11113215
11. Anderson, B. D., Barnes, A. N., Umar, S., Guo, X., Thongthum, T., & Gray, G. C. Reverse Zoonotic Transmission (Zooanthroponosis): An Increasing Threat to Animal Health. In Zoonoses: Infections Affecting Humans and Animals, *Cham: Springer International Publishing,* 2023, 1-63. https://doi.org/10.1007/978-3-030-85877-3_59-1
12. Annan, A., Baldwin, H. J., Corman, V. M., Klose, S. M., Owusu, M., Nkrumah, E. E., Badu, E. K., Anti, P., Agbenyega, O., Meyer, B., Oppong, S., Sarkodie, Y. A., Kalko, E. K. V., Lina, P. H. C., Godlevska, E. V., Reusken, C., Seebens, A., Gloza-Rausch, F., Vallo, P., … Drexler, J. F. Human betacoronavirus 2c EMC/2012-related viruses in bats, Ghana and Europe. *Emerging Infectious Diseases*, 2013, 19(3), 456–459. https://doi.org/10.3201/eid1903.121503
13. Ar Gouilh, M., Puechmaille, S. J., Diancourt, L., Vandenbogaert, M., Serra-Cobo, J., Lopez Roïg, M., Brown, P., Moutou, F., Caro, V., Vabret, A., & Manuguerra, J. C. SARS-CoV related Betacoronavirus and diverse Alphacoronavirus members found in western old-world. *Virology,* 2018, 517, 88–97. https://doi.org/10.1016/j.virol.2018.01.014
14. August, T. A., Mathews, F., & Nunn, M. A. Alphacoronavirus detected in bats in the United Kingdom. *Vector-Borne and Zoonotic Diseases*, 2012, 12(6), 530–533. https://doi.org/10.1089/vbz.2011.0829
15. Augusto, L.; Wek, R.C.; Sullivan, W.J. Host Sensing and Signal Transduction during Toxoplasma Stage Conversion. *Molecular Microbiology* 2021, 115, 839–848. https://doi.org/10.1111/mmi.14634
16. Baaert, L.; Wobus, C.E.; Van Coillie, E.; Thackray, L.B.; Debevere, J.; Uyttendaele, M. Detection of murine norovirus 1 by using plaque assay, transfection assay, and real-time reverse transcription-PCR before and after heat exposure. *Applied and Environmental Microbiology*, 2008, 74, 543–546. https://doi.org/10.1128/AEM.01039-07
17. Balboni, A., Palladini, A., Bogliani, G., & Battilani, M. Detection of a virus related to betacoronaviruses in Italian greater horseshoe bats. *Epidemiology and Infection*, 2011, 139(2), 216–219. https://doi.org/10.1017/S0950268810001147
18. Ballari, S.A.; Barrios-García, M.N. A Review of Wild Boar Sus Scrofa Diet and Factors Affecting Food Selection in Native and Introduced Ranges. *Mammal Review*, 2014, 44, 124–134. https://doi.org/doi:10.1111/mam.12015

19. Banerjee, A., Kulcsar, K., Misra, V., Frieman, M., & Mossman, K. Bats and coronaviruses. *Viruses*, 2019, 11(1), 7–9. https://doi.org/10.3390/v11010041
20. Bhattacharya, S., Sinha, S., Tilak, R., & Mardihusodo, S. J. The relationship between bats and human coronavirus: An exploratory review. *Journal of Health and Social Science*, 2020, 5(2), 219-230. https://doi.org/10.19204/2020/thrl7
21. Bier, N.S.; Stollberg, K.; Mayer-Scholl, A.; Johne, A.; Nöckler, K.; Richter, M. Seroprevalence of Toxoplasma Gondii in Wild Boar and Deer in Brandenburg, Germany. *Zoonoses and Public Health*, 2020, 67, 601–606. https://doi.org/10.1111/zph.12702
22. Bokaba, R.P.; Dermauw, V.; Morar-Leather, D.; Dorny, P.; Neves, L. Toxoplasma Gondii in African Wildlife: A Systematic Review. *Pathogens*, 2022, 11, 868. https://doi.org/10.3390/pathogens11080868
23. Bosco-Lauth, A. M.; Hartwig, A. E.; Porter, S. M.; Gordy, P. W.; Nehring, M.; Byas, A. D.; ... & Bowen, R. A. Experimental infection of domestic dogs and cats with SARS-CoV-2: Pathogenesis, transmission, and response to reexposure in cats. *Proceedings of the National Academy of Sciences*, 2020, 117(42), 26382-26388. https://doi.org/10.1073/pnas.2013102117
24. Brito, R. M. D. M., de Lima Bessa, G., Bastilho, A. L., Dantas-Torres, F., de Andrade-Neto, V. F., Bueno, L. L., ... & Magalhães, L. Genetic diversity of Toxoplasma gondii in South America: occurrence, immunity, and fate of infection. *Parasites & Vectors*, 2023, 16(1), 1-14. https://doi.org/10.1186/s13071-023-06080-w
25. Buesching, C.D.; Waterhouse, J.S.; Macdonald, D.W. Gas-Chromatographic Analyses of the Subcaudal Gland Secretion of the European Badger (Meles Meles) Part I: Chemical Differences Related to Individual Parameters. *Journal of Chemical Ecology*, 2002, 28, 41–56. https://doi.org/10.1023/A:1013558718057
26. Burrell, C. J., Howard, C. R., & Murphy, F. A. Coronaviruses. *Fenner and white's medical virology*, 2017, 437. https://doi.org/10.1016/B978-0-12-375156-0.00031-X
27. Burroughs, T., Knobler, S., & Lederberg, J. The emergence of zoonotic diseases: Understanding the impact on animal and human health: Workshop summary. Washington, DC*: National Academy Press, 2002.*
28. Calero-Bernal, R.; Saugar, J.M.; Frontera, E.; Pe´rez-Martín, J.E.; Habela, M.A.; Serrano, F.J.; Reina, D.; Fuentes, I. Prevalence and Genotype Identification of Toxoplasma Gondii in Wild Animals from Southwestern Spain. *Journal of Wildlife Diseases*, 2015, 51, 233–238. https://doi.org/10.7589/2013-09-233

29. Castillo-Contreras, R.; Mentaberre, G.; Fernandez Aguilar, X.; Conejero, C.; Colom-Cadena, A.; Ráez-Bravo, A.; González-Crespo, C.; Espunyes, J.; Lavín, S.; López-Olvera, J.R. Wild Boar in the City: Phenotypic Responses to Urbanisation. *Science of the Total Environment*, 2021, 773, 145593. https://doi.org/10.1016/j.scitotenv.2021.145593
30. Cholleti, H., Jong, J. de, Blomström, A.-L., & Berg, M. Characterization of Pipistrellus pygmaeus Bat Virome from Sweden. *Palliative Medicine*, 2022, 17(2), 107–108. https://doi.org/10.1191/0269216303pm716op
31. Conenna, I., Santini, L., Rocha, R., Monadjem, A., Cabeza, M., & Russo, D. Global patterns of functional trait variation along aridity gradients in bats. *Global Ecology and Biogeography*, 2021, 30(5), 1014-1029. https://doi.org/10.1111/geb.13278
32. Corman, V. M., Kallies, R., Philipps, H., Göpner, G., Müller, M. A., Eckerle, I., Brünink, S., Drosten, C., & Drexler, J. F. (2014). Characterization of a Novel Betacoronavirus Related to Middle East Respiratory Syndrome Coronavirus in European Hedgehogs. *Journal of Virology*, 88(1), 717–724. https://doi.org/10.1128/jvi.01600-13
33. Cox-Witton, K.; Baker, M. L.; Edson, D.; Peel, A. J.; Welbergen, J. A.; & Field, H. Risk of SARS-CoV-2 transmission from humans to bats–An Australian assessment. *One Health*, 2021, 13, 100247. https://doi.org/10.1016/j.onehlt.2021.100247
34. Crook, J. M., Murphy, I., Carter, D. P., Pullan, S. T., Carroll, M., Vipond, R., Cunningham, A. A., & Bell, D. (2021). Metagenomic identification of a new sarbecovirus from horseshoe bats in Europe. *Scientific Reports*, 11(1), 1–9. https://doi.org/10.1038/s41598-021-94011-z
35. Dakroub, H., Russo, D., Cistrone, L., Serra, F., Fusco, G., De Carlo, E., & Amoroso, M. G. (2022). A First Assessment of SARS-CoV-2 Circulation in Bats of Central–Southern Italy. *Pathogens,* 11(7), 742. https://doi.org/10.3390/pathogens11070742
36. De Benedictis, P., Marciano, S., Scaravelli, D., Priori, P., Zecchin, B., Capua, I., Monne, I., & Cattoli, G. (2014). Alpha and lineage C betaCoV infections in Italian bats. *Virus Genes*, 48(2), 366–371. https://doi.org/10.1007/s11262-013-1008-x
37. De Sabato, L., Lelli, D., Faccin, F., Canziani, S., Di Bartolo, I., Vaccari, G., & Moreno, A. (2019). Full genome characterization of two novel Alpha-coronavirus species from Italian bats. *Virus Research*, 260 (2018), 60–66. https://doi.org/10.1016/j.virusres.2018.11.007
38. De Sabato, L.; Amoroso, M.G.; Ianiro, G.; Esposito, C.; De Grossi, L.; Fusco, G.; Barone, A.; Martini, E.; Ostanello, F.; Di Bartolo, I. Detection of Hepatitis E Virus in Livers and Muscle Tissues of Wild Boars

in Italy. *Food and Environmental Virology,* 2020, 12, 1–8. https://doi.org/10.1007/s12560-019-09405-0

39. Delgado, I. L., Zúquete, S., Santos, D., Basto, A. P., Leitão, A., & Nolasco, S. The apicomplexan parasite Toxoplasma gondii. *Encyclopedia,* 2022, 2(1), 189-211. https://doi.org/10.3390/encyclopedia2010012
40. Destoumieux-Garzón, D., Mavingui, P., Boetsch, G., Boissier, J., Darriet, F., Duboz, P., ... & Voituron, Y. The one health concept: 10 years old and a long road ahead. *Frontiers in veterinary science,* 2018, 5, 14. https://doi.org/10.3389/fvets.2018.00014
41. Dietz, C.; von Helversen, O.; Nill, D.; Lina, P. H.; & Hutson, A. M. Bats of Britain, Europe, and Northwest Africa, *London: A & C Black*, 2009, 400.
42. Drexler, J. F., Corman, V. M., Wegner, T., Tateno, A. F., Zerbinati, R. M., Gloza-Rausch, F., Seebens, A., Müller, M. A., & Drosten, C. Amplification of emerging viruses in a bat colony. *Emerging Infectious Diseases,* 2011, 17(3), 449–456. https://doi.org/10.3201/eid1703.100526
43. Drexler, J. F., Gloza-Rausch, F., Glende, J., Corman, V. M., Muth, D., Goettsche, M., Seebens, A., Niedrig, M., Pfefferle, S., Yordanov, S., Zhelyazkov, L., Hermanns, U., Vallo, P., Lukashev, A., Müller, M. A., Deng, H., Herrler, G., & Drosten, C. Genomic Characterization of Severe Acute Respiratory Syndrome-Related Coronavirus in European Bats and Classification of Coronaviruses Based on Partial RNA-Dependent RNA Polymerase Gene Sequences. *Journal of Virology*, 2010, 84(21), 11336–11349. https://doi.org/10.1128/jvi.00650-10
44. Drosten, C.; Günther, S.; Preiser, W.; Van Der Werf, S.; Brodt, H. R.; Becker, S.; ... & Doerr, H. W. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *New England journal of medicine*, 2003, 348.20, 1967-1976. https://doi.org/10.1056/NEJMoa030747
45. Dubey, J.P. The History and Life Cycle of Toxoplasma Gondii. *Academic Press,* 2020, 1-19. https://doi.org/10.1016/B978-0-12-815041-2.00001-3
46. Dubey, J.P.; Jones, J.L. Toxoplasma Gondii Infection in Humans and Animals in the United States. *International Journal for Parasitology*, 2008, 38, 1257–1278. https://doi.org/10.1016/j.ijpara.2008.03.007
47. Elmore, S.A.; Jones, J.L.; Conrad, P.A.; Patton, S.; Lindsay, D.S.; Dubey, J.P. Toxoplasma Gondii: Epidemiology, Feline Clinical Aspects, and Prevention. *Trends in Parasitology,* 2010, 26, 190–196. https://doi.org/10.1016/j.pt.2010.01.009
48. Erkyihun, G. A., & Alemayehu, M. B. One Health approach for the control of zoonotic diseases. *Zoonoses, 2022.* https://doi.org/10.15212/ZOONOSES-2022-0037

49. Escudero-Pérez, B., Lalande, A., Mathieu, C., & Lawrence, P. Host–Pathogen interactions influencing zoonotic spillover potential and transmission in humans. *Viruses,* 2023, 15(3), 599. https://doi.org/10.3390/v15030599

50. Esteves, F.; Aguiar, D.; Rosado, J.; Costa, M.L.; de Sousa, B.; Antunes, F.; Matos, O. Toxoplasma Gondii Prevalence in Cats from Lisbon and in Pigs from Centre and South of Portugal. *Veterinary Parasitology* 2014, 200, 8–12. https://doi.org/10.1016/j.vetpar.2013.12.017

51. Fagre, A. C.; Cohen, L. E.; Eskew, E. A.; Farrell, M.; Glennon, E.; Joseph, M. B.; ... & Albery, G. F. Assessing the risk of human-to-wildlife pathogen transmission for conservation and public health. *Ecology Letters*, 2022, 00,1-16. https://doi.org/10.1111/ele.14003

52. Falcón, A., Vázquez-Morón, S., Casas, I., Aznar, C., Ruiz, G., Pozo, F., Perez-Breña, P., Juste, J., Ibáñez, C., Garin, I., Aihartza, J., & Echevarría, J. E. Detection of alpha and betacoronaviruses in multiple Iberian bat species. *Archives of Virology*, 2011, 156(10), 1883–1890. https://doi.org/10.1007/s00705-011-1057-1

53. Fan, Y., Zhao, K., Shi, Z. L., & Zhou, P. Bat coronaviruses in China. *Viruses*, 2019, 11(3), 27–32. https://doi.org/10.3390/v11030210

54. Farooq, R., Majid, S., Hanif, A., & Qureshi, W. Current insights into the genomics of novel coronavirus: SARS-Cov-2. *EC Clin Med Case Rep*, 2020, 3, 3-11.

55. Ferroglio, E.; Bosio, F.; Trisciuoglio, A.; Zanet, S. Toxoplasma Gondii in Sympatric Wild Herbivores and Carnivores: Epidemiology of Infection in the Western Alps. *Parasites and Vectors*, 2014, 7, 2–5. https://doi.org/10.1186/1756-3305-7-196

56. Fischer, K., Zeus, V., Kwasnitschka, L., Kerth, G., Haase, M., Groschup, M. H., & Balkema-Buschmann, A. Insectivorous bats carry host specific astroviruses and coronaviruses across different regions in Germany. *Infection, Genetics and Evolution*, 2016, 37, 108–116. https://doi.org/10.1016/j.meegid.2015.11.010

57. Formenti, N.; Trogu, T.; Pedrotti, L.; Gaffuri, A.; Lanfranchi, P.; Ferrari, N. Toxoplasma Gondii Infection in Alpine Red Deer (Cervus Elaphus): Its Spread and Effects on Fertility. *PLoS ONE,* 2015, 10, 1–11. https://doi.org/10.1371/journal.pone.0138472

58. Frick, W. F.; Kingston, T.; & Flanders, J. A review of the major threats and challenges to global bat conservation. *Annals of the New York Academy of Sciences*, 2020, 1469.1, 5-25. https://doi.org/10.1111/nyas.14045

59. Fusco, G.; Rinaldi, L.; Guarino, A.; Proroga, Y.T.R.; Pesce, A.; Giuseppina, D.M.; Cringoli, G. Toxoplasma Gondii in Sheep from the Campania Region (Italy). *Veterinary Parasitology*, 2007, 149, 271–274. https://doi.org/10.1016/j.vetpar.2007.07.020

60. Gazzonis, A. L., Villa, L., Lubian, E., Ressegotti, S., Grilli, G., Raimondi, S., ... & Manfredi, M. T. Molecular Survey on Toxoplasma gondii and Neospora caninum infection in wild birds of prey admitted to recovery centers in Northern Italy. *Microorganisms,* 2021, 9(4), 736. https://doi.org/10.3390/microorganisms9040736

61. Gering, E.; Laubach, Z.M.; Weber, P.S.D.; Soboll Hussey, G.; Lehmann, K.D.S.; Montgomery, T.M.; Turner, J.W.; Perng, W.; Pioon, M.O.; Holekamp, K.E.; et al. Toxoplasma Gondii Infections Are Associated with Costly Boldness toward Felids in a Wild Host. *Nature Communications*, 2021, 12. https://doi.org/10.1038/s41467-021-24092-x

62. Gibson, JF; Kelso, S.; & Skevington, JH. Band-cutting no more: A method for the isolation and purification of target PCR bands from multiplex PCR products using new technology. *Molecular Phylogenetics and Evolution,* 2010, 56.3, 1126-1128. https://doi.org/10.1016/j.ympev.2010.05.005

63. Gloza-rausch, F., Ipsen, A., Seebens, A., Göttsche, M., Panning, M., Drexler, J. F., Petersen, N., Annan, A., Grywna, K., Müller, M., Pfefferle, S., & Drosten, C. Detection and Prevalence Patterns of Group I Coronaviruses in Bats, Northern Germany, *Emerging Infectious Diseases*, 2008, 14(4). https://doi.org/10.3201/eid1404.071439

64. Goffard, A., Demanche, C., Arthur, L., Pinçon, C., Michaux, J., & Dubuisson, J. Alphacoronaviruses detected in french bats are phylogeographically linked to coronaviruses of european bats. *Viruses,* 2015, 7(12), 6279–6290. https://doi.org/10.3390/v7122937

65. Gomes, B. M., Rebelo, C. B., & de Sousa, L. A. Public health, surveillance systems and preventive medicine in an interconnected world. In One Health. *Academic Press*, 2022, 33-71. https://doi.org/10.1016/B978-0-12-822794-7.00006-X

66. Gryseels, S.; De Bruyn, L.; Gyselings, R.; Calvignac-Spencer, S.; Leendertz, F. H.; & Leirs, H. Risk of human-to-wildlife transmission of SARS-CoV-2. *Mammal Review,* 2021, 51(2), 272-292. https://doi.org/10.1111/mam.12225

67. Guarner, J. Three Emerging Coronaviruses in Two Decades: The Story of SARS, MERS, and Now COVID-19. *American Journal of Clinical Pathology*, 2020, 153(4), 420–421. https://doi.org/10.1093/ajcp/aqaa029

68. Hall, J. S.; Knowles, S.; Nashold, S. W.; Ip, H. S.; Leon, A. E.; Rocke, T.; ... & Hofmeister, E. Experimental challenge of a North American bat species, big brown bat (Eptesicus fuscus), with SARS-CoV-2. *Transboundary and emerging diseases,* 2021, 68.6, 3443-3452. https://doi.org/10.1111/tbed.13949

69. Herrero, L.; Gracia, M.J.; Pérez-Arquillué, C.; Lázaro, R.; Herrera, M.; Herrera, A.; Bayarri, S. Toxoplasma Gondii: Pig Seroprevalence, Associated Risk Factors and Viability in Fresh Pork Meat. *Veterinary Parasitology,* 2016, 224, 52–59. https://doi.org/10.1016/j.vetpar.2016.05.010

70. Holmes, EC; Goldstein, SA; Rasmussen, AL; Robertson, DL; Crits-Christoph, A.; Wertheim, JO; ... &Rambaut, A. The origins of SARS-CoV-2: *A critical review. Cell*, 2021, 184(19), 4848-4856. https://doi.org/10.1016/j.cell.2021.08.017

71. Hu, B.; Zeng, L. P.; Yang, X. L.; Ge, X. Y.; Zhang, W.; Li, B.; ... & Shi, Z. L. Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus. *PLoS pathogens,* 2017, 13.11, e1006698. https://doi.org/10.1371/journal.ppat.1006698

72. Hussain, K., Ijaz, M., Rabbani, A. H., Ali, A., & Khan, Y. R. Reverse zoonosis and animal health. *Veterinary Pathobiology and Public Health*, 2021, 493-504. https://doi.org/10.47278/book.vpph/2021.042

73. Hutson, Michael, A., & Mickleburgh, S. P. Microchiropteran bats: global status survey and conservation action plan. *IUCN*, 2001, 56.

74. Irving, A. T., Ahn, M., Goh, G., Anderson, D. E., & Wang, L. F. Lessons from the host defences of bats, a unique viral reservoir. *Nature*, 2021, 589(7842), 363-370. https://doi.org/10.1038/s41586-020-03128-0

75. Islam, A.; Ferdous, J.; Islam, S.; Sayeed, M. A.; Rahman, M. K.;Saha, O.; Hassan, M.M.; & Shirin, T. Transmission dynamics and susceptibility patterns of SARS-CoV-2 in domestic, farmed, and wild animals: Sustainable One Health surveillance for conservation and public health to prevent future epidemics and pandemics. *Transboundary and emerging diseases*, 2021, 1-21. https://doi.org/10.1111/tbed.14356

76. Jahangir, M. A., Rizvi, A. M., & Fatima, M. Coronavirus (COVID-19): History, Current Knowledge and Pipeline Medications. *International Journal of Pharmaceutics & Pharmacology*, 2020, 4(1), 1–9. https://doi.org/10.31531/2581-3080.1000140

77. Jemeršić, L.; Lojkić, I.; Krešić, N.; Keros, T.; Zelenika, T. A.; Jurinović, L.; ... & Brnić, D. Investigating the presence of SARS CoV-2 in free-living and captive animals. *Pathogens*, 2021, 10(6), 635. https://doi.org/10.3390/pathogens10060635

78. Joffrin, L., Hoarau, A. O. G., Lagadec, E., Torrontegi, O., Köster, M., Le Minter, G., Dietrich, M., Mavingui, P., & Lebarbenchon, C. Seasonality of coronavirus shedding in tropical bats. *Royal Society open science,* 2022, 9(2), 211600. https://doi.org/10.1098/rsos.211600

79. Johnson, C. K., Hitchens, P. L., Pandit, P. S., Rushmore, J., Evans, T. S., Young, C. C. W., & Doyle, M. M. Global shifts in mammalian population trends reveal key predictors of virus spillover risk. *Proceedings of the Royal Society B: Biological Sciences,* 2020, 287(1924). https://doi.org/10.1098/rspb.2019.2736

80. Johnson, S. K., & Johnson, P. T. Toxoplasmosis: recent advances in understanding the link between infection and host behavior. *Annual Review of Animal Biosciences*, 2021, 9, 249-264. https://doi.org/10.1146/annurev-animal-081720-111125

81. Jones, S.; Bell, T.; Coleman, C.M.; Harris, D.; Woodward, G.; Worledge, L.; Roberts, H.; McElhinney, L.; Aegerter, J.; Ran-some, E.; et al. Testing bats in rehabilitation for SARS-CoV-2 before release into the wild. *Conservation Science and Practice*, 2022, e12707. https://doi.org/10.1111/csp2.12707

82. Jung, K., & Kalko, E. K. V. Adaptability and vulnerability of high-flying Neotropical aerial insectivorous bats to urbanization. *Diversity and Distributions*, 2011, 17(2), 262–274. https://doi.org/10.1111/j.1472-4642.2010.00738.x

83. Kemenesi, G., Dallos, B., Görföl, T., Boldogh, S., Estók, P., Kurucz, K., Kutas, A., Földes, F., Oldal, M., Németh, V., Martella, V., Bányai, K., & Jakab, F. Molecular survey of RNA viruses in hungarian bats: Discovering novel astroviruses, coronaviruses, and caliciviruses. *Vector-Borne and Zoonotic Diseases,* 2014, 14(12), 846–855. https://doi.org/10.1089/vbz.2014.1637

84. Kenney, S. P.; Wang, Q.; Vlasova, A.; Jung, K.; & Saif, L. Naturally occurring animal coronaviruses as models for studying highly pathogenic human coronaviral disease. *Veterinary pathology*, 2021, 58.3, 438-452. https://doi.org/10.1177/0300985820980842

85. Kesheh, M. M., Hosseini, P., Soltani, S., & Zandi, M. An overview on the seven pathogenic human coronaviruses. Reviews in Medical Virology, 2022, 32(2). https://doi.org/10.1002/rmv.2282

86. Kivisto, I., Tidenberg, E., Lilley, T., Suominen, K., Forbes, K. M., Vapalahti, O., Huovilainen, A., & Sironen, T. First Report of Coronaviruses in Northern European Bats. 2020, 20(2), 155–158. https://doi.org/10.1089/vbz.2018.2367

87. Kobayashi, S.; Shimizu, Y.; Yamamoto, T.; Hayama, Y.; Yamaguchi, E.; Hanafusa, Y.; Osaki, M. First Nationwide Survey of the Seroprevalence of Toxoplasma Gondii in Wild Boars in Japan. 2021. https://doi.org/10.1007/s00436-021-07086-y

88. Kohl, C., & Kurth, A. European bats as carriers of viruses with zoonotic potential. *Viruses,* 2014, 6(8), 3110–3128. https://doi.org/10.3390/v6083110

89. Kohl, C., Nitsche, A., & Kurth, A. Update on potentially zoonotic viruses of European bats. *Vaccines,* 2021, 9(7), 690. https://doi.org/10.3390/vaccines9070690
90. Krammer, Florian. SARS-CoV-2 vaccines in development. *Nature,* 2020, 586(7830), 516-527. https://doi.org/10.1038/s41586-020-2798-3
91. Kumakamba, C., Niama, F. R., Muyembe, F., Mombouli, J. V., Kingebeni, P. M., Nina, R. A., Lukusa, I. N., Bounga, G., N'Kawa, F., Nkoua, C. G., Losoma, J. A., Mulembakani, P., Makuwa, M., Tamufe, U., Gillis, A., Lebreton, M., Olson, S. H., Cameron, K., Reed, P., … Lange, C. E. Coronavirus surveillance in wildlife from two Congo basin countries detects RNA of multiple species circulating in bats and rodents. *PLoS ONE,* 2021, 16(6), 1–17. https://doi.org/10.1371/journal.pone.0236971
92. Kunz, T. H.; Braun de Torrez, E.; Bauer, D.; Lobova, T.; & Fleming, T. H. Ecosystem services provided by bats. *Annals of the New York Academy of Sciences*, 2011, 1223(1), 1-38. https://doi.org/10.1111/j.1749-6632.2011.06004.x
93. Laguna, E.; Barasona, J.A.; Vicente, J.; Keuling, O.; Acevedo, P. Differences in Wild Boar Spatial Behaviour among Land Uses and Management Scenarios in Mediterranean Ecosystems. *Science of the Total Environment*, 2021, 796. https://doi.org/10.1016/j.scitotenv.2021.148966
94. Lazov, C. M., Belsham, G. J., Bøtner, A., & Rasmussen, T. B. (2021). Full-genome sequences of alphacoronaviruses and astroviruses from myotis and pipistrelle bats in Denmark. *Viruses*, 13(6), 1–20. https://doi.org/10.3390/v13061073
95. Lazov, C. M., Chriél, M., Baagøe, H. J., Fjederholt, E., Deng, Y., Kooi, E. A., Belsham, G. J., Bøtner, A., & Rasmussen, T. B. Detection and characterization of distinct alphacoronaviruses in five different bat species in Denmark. Viruses, 2018, 10(9). https://doi.org/10.3390/v10090486
96. Lecis, R., Mucedda, M., Pidinchedda, E., Pittau, M., & Alberti, A. Molecular identification of Betacoronavirus in bats from Sardinia (Italy): first detection and phylogeny. *Virus Genes*, 2019, 55(1), 60–67. https://doi.org/10.1007/s11262-018-1614-8
97. Lelli, D., Papetti, A., Sabelli, C., Rosti, E., Moreno, A., & Boniotti, M. B. Detection of coronaviruses in bats of various species in Italy. *Viruses,* 2013, 5(11), 2679–2689. https://doi.org/10.3390/v5112679
98. Leopardi, S.; Barneschi, E.; Manna, G.; Zecchin, B.; Priori, P.;Drzewniokova, P.; ... & Benedictis, P. D. Spillover of West Caucasian Bat Lyssavirus (WCBV) in a Domestic Cat and Westward Expansion in the Palearctic Region*. Viruses*, 2021, 13.10, 2064. https://doi.org/10.3390/v13102064
99. Logan, A. C., Berman, S. H., Berman, B. M., & Prescott, S. L. Healing anthropocene syndrome: planetary health requires remediation of the toxic post-truth environment. *Challenges*, 2021, 12(1), 1. https://doi.org/10.3390/challe12010001

100. Louhimies, S. Directive 86/609/EEC on the Protection of Animals Used for Experimental and Other Scientific Purposes. *ATLA Alternatives to Laboratory Animals,* 2002, 30, 217–219. https://doi.org/10.1177/026119290203002s36

101. Lwande, O. W., Thalin, T., de Jong, J., Sjödin, A., Näslund, J., Evander, M., & Ecke, F. Alphacoronavirus in a Daubenton's Myotis Bat (Myotis daubentonii) in Sweden. *Viruses,* 2022, 14(3), 1–10. https://doi.org/10.3390/v14030556

102. Mackenzie, J. S., Jeggo, M., Daszak, P., & Richt, J. A. (Eds.). One Health: The human-animal-environment interfaces in emerging infectious diseases. *Berlin: Springer*, 2013, 366. https://doi.org/10.1007/978-3-642-36889-9

103. Medellín, I.H.; Racey, P.; Shapiro, J.T.; Vicente-Santos, A.; Viquez-R, L.; Worledge, L. IUCN SSC Bat Specialist Group (BSG) recommendations to reduce the risk of transmission of SARS-CoV-2 from humans to bats by cavers. MAP: Minimize, Assess, Protect. *IUCN SSC Bat Spec. Group*, 2020, 1–10. Available online: https://iucnbsg.org/uploads/6/5/0/9/6509077/map_recommendations_cavers_v2_dec_2021.pdf (accessed on 22 December 2021).

104. Meriggi, A.; Brangi, A.; Schenone, L.; Signorelli, D.; Milanesi, P. Changes of Wolf (Canis Lupus) Diet in Italy in Relation to the Increase of Wild Ungulate Abundance. *Ethology Ecology and Evolution*, 2011, 23, 195–210. https://doi.org/10.1080/03949370.2011.577814

105. Milne, G.; Webster, J.P.; Walker, M. Toxoplasma Gondii: An Underestimated Threat? *Trends in Parasitology*, 2020, 36, 959–969. https://doi.org/10.1016/j.pt.2020.08.005

106. Mishra, J., Mishra, P., & Arora, N. K. Linkages between environmental issues and zoonotic diseases: with reference to COVID-19 pandemic. *Environmental Sustainability*, 2021, 4(3), 455-467. https://doi.org/10.1007/s42398-021-00165-x

107. Mols, V. C., Lamers, M. M., Leijten, L. M., Breugem, T. I., van de Bildt, M. W., van den Doel, P. B., ... & Begeman, L. Intestinal Tropism of a Betacoronavirus (Merbecovirus) in Nathusius's Pipistrelle Bat (Pipistrellus nathusii), Its Natural Host. *Journal of virology*, 2023, 97(3). https://doi.org/10.1128/jvi.00099-23

108. Morales-González, A.; Fernández-Gil, A.; Quevedo, M.; Revilla, E. Patterns and Determinants of Dispersal in Grey Wolves (Canis Lupus). *Biological Reviews*, 2022, 97, 466–480. https://doi.org/10.1111/brv.12807

109. Moreno, A., Lelli, D., De Sabato, L., Zaccaria, G., Boni, A., Sozzi, E., Prosperi, A., Lavazza, A., Cella, E., Castrucci, M. R., Cicozzi, M., & Vaccari, G. Detection and full genome characterization of two beta

CoV viruses related to Middle East respiratory syndrome from bats in Italy. *Virology Journal*, 2017, 14(1), 1–11. https://doi.org/10.1186/s12985-017-0907-1

110. Mori, E.; Benatti, L.; Lovari, S.; Ferretti, F. What Does the Wild Boar Mean to the Wolf? *European Journal of Wildlife Research*, 2017, 63, 1–5. https://doi.org/10.1007/s10344-016-1060-7.
111. Mubareka, S., Amuasi, J., Banerjee, A., Carabin, H., Copper Jack, J., Jardine, C., ... & Jane Parmley, E. Strengthening a One Health approach to emerging zoonoses. *Facets,* 2023, 8(1), 1-64. https://doi.org/10.1139/facets-2021-0190
112. Munasinghe, M. COVID-19 and sustainable development. *International Journal of Sustainable Development*, 2020, 23(1-2), 1-24. https://doi.org/10.1504/IJSD.2020.112182
113. Nabi, G., Wang, Y., Lü, L., Jiang, C., Ahmad, S., Wu, Y., & Li, D. Bats and birds as viral reservoirs: A physiological and ecological perspective. Science of the Total Environment, 2021, 754, 142372. https://doi.org/10.1016/j.scitotenv.2020.142372
114. Nassar, A., Ibrahim, I. M., Amin, F. G., Magdy, M., Elgharib, A. M., Azzam, E. B., Nasser, F., Yousry, K., Shamkh, I. M., Mahdy, S. M., & Elfiky, A. A. A review of human coronaviruses' receptors: The host-cell targets for the crown bearing viruses. *Molecules*, 2021, 26(21), 1–24. https://doi.org/10.3390/molecules26216455
115. Natoli, E.; Maragliano, L.; Cariola, G.; Faini, A.; Bonanni, R.; Cafazzo, S.; Fantini, C. Management of Feral Domestic Cats in the Urban Environment of Rome (Italy). *Preventive Veterinary Medicine,* 2006, 77, 180–185. https://doi.org/10.1016/j.prevetmed.2006.06.005
116. Nuñez, G. B.; Cunningham, A.; Moise, E.; Fils, B.; Frick, W.; Islam, N.; & Dinh, T. V. IUCN SSC Bat Specialist Group (BSG) Recommended Strategy for Researchers to Reduce the Risk of Transmission of SARS-CoV-2 from Humans to Bats. *IUCN SSC Bat Specialist Group*, 2020.
117. Olival, K. J., Cryan, P. M., Amman, B. R., Baric, R. S., Blehert, D. S., Brook, C. E., ... & Wang, L. F. Possibility for reverse zoonotic transmission of SARS-CoV-2 to free-ranging wildlife: A case study of bats. PLoS pathogens, 2020, 16(9). https://doi.org/10.1371/journal.ppat.1008758
118. Orłowska, A., Smreczak, M., Thor, K., Niedbalska, M., Pawelec, D., Trębas, P., & Rola, J. The Genetic Characterization of the First Detected Bat Coronaviruses in Poland Revealed SARS-Related Types and Alphacoronaviruses. *Viruses*, 2022, 14(9). https://doi.org/10.3390/v14091914
119. Ossi, F.; Ranc, N.; Moorcroft, P.; Bonanni, P.; Cagnacci, F. Ecological and Behavioral Drivers of Supplemental Feeding Use by Roe Deer Capreolus Capreolus in a Peri-Urban Context. *Animals*, 2020, 10, 1–14. https://doi.org/10.3390/ani10112088

120. Pauly, M., Pir, J. B., Loesch, C., Sausy, A., Snoeck, C. J., Hübschen, J. M., & Muller, C. P. Novel alphacoronaviruses and paramyxoviruses cocirculate with type 1 and severe acute respiratory system (SARS)-related betacoronaviruses in synanthropic bats of Luxembourg. *Applied and Environmental Microbiology*, 2017, 83(18). https://doi.org/10.1128/AEM.01326-17
121. Pepe, P.; Bosco, A.; Capuano, F.; Baldi, L.; Giordano, A.; Mancusi, A.; Buonanno, M.; Morena, L.; Pinto, R.; Sarnelli, P.; et al. Towards an Integrated Approach for Monitoring Toxoplasmosis in Southern Italy. *Animals*, 2021, 11, 1–12. https://doi.org/10.3390/ani11071949
122. Perkins, S. E., Cattadori, I., & Hudson, P. J. The role of mammals in emerging zoonoses. *Mammal Study*, 2005, 30(Supplement), S67-S71. https://doi.org/10.3106/1348-6160(2005)30[S67:TROMIE]2.0.CO;2
123. Phelps, K. L., Hamel, L., Alhmoud, N., Ali, S., Bilgin, R., Sidamonidze, K., ... & Olival, K. J. Bat research networks and viral surveillance: gaps and opportunities in Western Asia. *Viruses*, 2019, 11(3), 240. https://doi.org/10.3390/v11030240
124. Pigozzi, G. The Diet of the European Badger in a Mediterranean Coastal Area. *Acta Theriologica*, 1991, 36, 293–306. https://doi.org/10.4098/at.arch.91-31
125. Poudel, U., Subedi, D., Pantha, S., & Dhakal, S. Animal coronaviruses and coronavirus disease 2019: Lesson for one health approach. *Open Veterinary Journal*, 2020, 10(3), 239–251. https://doi.org/10.4314/ovj.v10i3.1
126. Prasad, N., Gopalakrishnan, N., Sahay, M., Gupta, A., & Agarwal, S. Epidemiology, genomic structure, the molecular mechanism of injury, diagnosis and clinical manifestations of coronavirus infection: An overview. *Indian Journal of Nephrology,* 2020, 30(3), 143. https://doi.org/10.4103/ijn.IJN_191_20
127. Queenan, K., Garnier, J., Nielsen, L. R., Buttigieg, S., Meneghi, D. D., Holmberg, M., ... & Kock, R. Roadmap to a One Health agenda 2030. *CABI Reviews*, 2017, 1-17. https://doi.org/10.1079/PAVSNNR201712014
128. Racey, P. A. Reproductive assessment of bats. In T. H. Kunz & S. Parsons (Eds.), Ecological and behavioral methods for the study of bats. *The John Hopkins University Press*, 2009, 249-264.
129. Reiling, S.; Dixon, B. Toxoplasma Gondii: How an Amazonian Parasite Became an Inuit Health Issue 2019. https://doi.org/10.4745/ccdr.v45i78a03
130. Reusken, C. B. E. M., Lina, P. H. C., Pielaat, A., de Vries, A., Dam-Deisz, C., Adema, J., Drexler, J. F., Drosten, C., & Kooi, E. A. (2010). Circulation of group 2 coronaviruses in a bat species common to urban areas in Western Europe. *Vector Borne and Zoonotic Diseases (Larchmont, N.Y.)*, 2010, 0(8), 785–791. https://doi.org/10.1089/vbz.2009.0173

131. Rihtarič, D., Hostnik, P., Steyer, A., Grom, J., & Toplak, I. Identification of SARS-like coronaviruses in horseshoe bats (Rhinolophus hipposideros) in Slovenia*. Archives of Virology*, 2010, 155(4), 507–514. https://doi.org/10.1007/s00705-010-0612-5

132. Rizzo, F., Edenborough, K. M., Toffoli, R., Culasso, P., Zoppi, S., Dondo, A., Robetto, S., Rosati, S., Lander, A., Kurth, A., Orusa, R., Bertolotti, L., & Mandola, M. L. Coronavirus and paramyxovirus in bats from Northwest Italy. *BMC Veterinary Research*, 2017, 13(1), 1–11. https://doi.org/10.1186/s12917-017-1307-x

133. Rostami, A.; Riahi, S.M.; Fakhri, Y.; Saber, V.; Hanifehpour, H.; Valizadeh, S.; Gholizadeh, M.; Pouya, R.H.; Gamble, H.R. The Global Seroprevalence of Toxoplasma Gondii among Wild Boars: A Systematic Review and Meta-Analysis. *Veterinary Parasitology*, 2017, 244, 12–20. https://doi.org/10.1016/j.vetpar.2017.07.013

134. Rodriguez Franco, C., & Conje, J. The evolution of the dialogue and perspectives on sustainable forest management with special emphasis on the United States of America. *Journal of Sustainable Forestry*, 2023, 42(8), 747-791. https://doi.org/10.1080/10549811.2022.2059687

135. Ruiz-Aravena, M., McKee, C., Gamble, A., Lunn, T., Morris, A., Snedden, C. E., Yinda, C. K., Port, J. R., Buchholz, D. W., Yeo, Y. Y., Faust, C., Jax, E., Dee, L., Jones, D. N., Kessler, M. K., Falvo, C., Crowley, D., Bharti, N., Brook, C. E., … Plowright, R. K. Ecology, evolution and spillover of coronaviruses from bats. *In Nature Reviews Microbiology*, 2022, 20(5). Springer US. https://doi.org/10.1038/s41579-021-00652-2

136. Russo, D.; & Ancillotto, L. Sensitivity of bats to urbanization: a review. *Mammalian Biology*, 2015, 80(3), 205-212. https://doi.org/10.1016/j.mambio.2014.10.003

137. Saeed, O. S.; El-Deeb, A. H.; & Hussein Ahmed, H. A. No evidence of SARS-CoV-2 infection in Rousettus aegyptiacus bat in Egypt. *International Journal of Veterinary Science and Medicine*, 2021, 9(1), 59-61. https://doi.org/10.1080/23144599.2021.1991135

138. Saïd, S.; Tolon, V.; Brandt, S.; Baubet, E. Sex Effect on Habitat Selection in Response to Hunting Disturbance: The Study of Wild Boar. *European Journal of Wildlife Research*, 2012, 58, 107–115. https://doi.org/10.1007/s10344-011-0548-4

139. Sakban, F.M.; A'aiz, N.N. Investigate the Toxoplasma Gondii Infection in the Consumed Beef in Al-Diwaniyah Province. *Iraqi Journal of Veterinary Sciences*, 2020, 34, 95–99. https://doi.org/10.33899/IJVS.2020.164336.

140. Salinas-Ramos, V.B.; Mori, E.; Bosso, L.; Ancillotto, L.; Russo, D. Zoonotic Risk: One More Good Reason Why Cats Should Be Kept Away from Bats. *Pathogens,* 2021, 10, 1–13. https://doi.org/10.3390/pathogens10030304

141. Schlottau, K.; Rissmann, M.; Graaf, A.; Schön, J.; Sehl, J.; Wylezich, C.; ... & Beer, M. SARS-CoV-2 in fruit bats, ferrets, pigs, and chickens: an experimental transmission study. *The Lancet Microbe*, 2020, 1.5, e218-e225. https://doi.org/10.1016/S2666-5247(20)30089-6

142. Serra-Cobo, J., & Lopez-Roig, M. Bats and emerging infections: an ecological and virological puzzle. *Emerging and Re-emerging Viral Infections: Advances in Microbiology, Infectious Diseases and Public Health,* 2017, 6, 35-48. https://doi.org/10.1007/5584_2016_131

143. Sgroi, G.; Viscardi, M.; Santoro, M.; Borriello, G.; D'Alessio, N.; Boccia, F.; Pacifico, L.; Fioretti, A.; Veneziano, V.; Fusco, G. Genotyping of Toxoplasma Gondii in Wild Boar (Sus Scrofa) in Southern Italy: Epidemiological Survey and Associated Risk for Consumers. *Zoonoses and Public Health,* 2020, 67, 805–813. https://doi.org/10.1111/zph.12762

144. Shapiro, K.; Bahia-Oliveira, L.; Dixon, B.; Dumètre, A.; de Wit, L.A.; VanWormer, E.; Villena, I. Environmental Transmission of Toxoplasma Gondii: Oocysts in Water, Soil and Food. *Food and Waterborne Parasitology*, 2019, 15. https://doi.org/10.1016/j.fawpar.2019.e00049

145. Sharan, M., Vijay, D., Yadav, J. P., Bedi, J. S., & Dhaka, P. Surveillance and Response Strategies for Zoonotic Diseases: A Comprehensive Review. *Science in One Health*, 2023, 100050. https://doi.org/10.1016/j.soh.2023.100050

146. Skonhoft, A.; Friberg, V. Optimal Harvesting in the Presence of Predation: An Age-Structured Modelling Approach. *Journal of Environmental Management,* 2021, 277, 111341. https://doi.org/10.1016/j.jenvman.2020.111341

147. Smith, D. D., & Frenkel, J. Prevalence of antibodies to Toxoplasma gondii in wild mammals of Missouri and east central Kansas: biologic and ecologic considerations of transmission. *Journal of Wildlife Diseases*, 1995, 31(1), 15-21. https://doi.org/10.7589/0090-3558-31.1.15

148. Sofi, O. M. U. D., Sofi, Tt. A., Bandroo, G., Javid, F., Nabi, B., & Sheikh, A. A. Possible Collaboration of Veterinarians and Medicos over the Fight against Covid-19 with "One-Health Approach." *Virology & Mycology 1 Virol Mycol Open Access*, 2021, 10(1), 1–5.

149. Sousa, R.Á. de; Lemos, J. da F.; Farias, L.A.; Lopes, C.D.; Santos, K.R. dos Seroprevalence and Risk Factors for Toxoplasma Gondii Infection in Pigs in Southern Piauí. *Revista Brasileira de Parasitologia Veterinária*, 2014, 23, 98–100. https://doi.org/10.1590/s1984-29612014015

150. Stelzer, S.; Basso, W.; Benavides Silván, J.; Ortega-Mora, L.M.; Maksimov, P.; Gethmann, J.; Conraths, F.J.; Schares, G. Toxoplasma Gondii Infection and Toxoplasmosis in Farm Animals: Risk Factors and Economic Impact. *Food and Waterborne Parasitology*, 2019, 15. https://doi.org/10.1016/j.fawpar.2019.e00037
151. Sullivan Jr, W. J., & Jeffers, V. Mechanisms of Toxoplasma gondii persistence and latency. *FEMS microbiology reviews,* 2012, 36(3), 717-733. https://doi.org/10.1111/j.1574-6976.2011.00305.x
152. Tajudeen, Y. A., Oladipo, H. J., Oladunjoye, I. O., Mustapha, M. O., Mustapha, S. T., Abdullahi, A. A., ... & El-Sherbini, M. S. Preventing the Next Pandemic through a Planetary Health Approach: A Focus on Key Drivers of Zoonosis. *Challenges*, 2022, 13(2), 50. https://doi.org/10.3390/challe13020050
153. Tan, C. C. S., Trew, J., Peacock, T. P., Mok, K. Y., Hart, C., Lau, K., Ni, D., Orme, C. D. L., Ransome, E., Pearse, W. D., Coleman, C. M., Bailey, D., Thakur, N., Quantrill, J. L., Sukhova, K., Richard, D., Kahane, L., Woodward, G., Bell, T., … Savolainen, V. Genomic screening of 16 UK native bat species through conservationist networks uncovers coronaviruses with zoonotic potential. *Nature Communications,* 2023, 14(1), 1–45. https://doi.org/10.1038/s41467-023-38717-w
154. Temmam, S.;Vongphayloth, K.; Baquero, E.; Munier, S.; Bonomi, M.; Regnault, B.; ... &Eloit, M. Bat coronaviruses related to SARS-CoV-2 and infectious for human cells. *Nature*, 2022, 1.7, 330-336. https://doi.org/10.1038/s41586-022-04532-4
155. Tiwari, R., Dhama, K., Sharun, K., Iqbal Yatoo, M., Malik, Y. S., Singh, R., Michalak, I., Sah, R., Bonilla-Aldana, D. K., & Rodriguez-Morales, A. J. COVID-19: animals, veterinary and zoonotic links. *Veterinary Quarterly*, 2020, 40(1), 169–182. https://doi.org/10.1080/01652176.2020.1766725
156. Tofani, S.; Ianiro, G.; De Sabato, L.; Monini, M.; Angeloni, G.; Ponterio, E.; D'Agostino, C.; Di Bari, M.A.; Valeri, M.; Di Bartolo, I. Detection and whole genome sequencing of murine norovirus in animal facility in Italy. *Anim. Biotechnol*. 2022, 33, 1142–1149. https://doi.org/10.1080/10495398.2021.1876081
157. Torretta, E.; Corradini, A.; Pedrotti, L.; Bani, L.; Bisi, F.; Dondina, O. Hide-and-Seek in a Highly Human-Dominated Landscape: Insights into Movement Patterns and Selection of Resting Sites of Rehabilitated Wolves (Canis Lupus) in Northern Italy. 2022. https://doi.org/10.3390/ani13010046
158. Torrey, E.F.; Yolken, R.H. Toxoplasma Gondii and Schizophrenia. *Emerging Infectious Diseases* 2003, 9, 1375–1380. https://doi.org/10.3201/eid0911.030143
159. Vandegrift, K. J., Wale, N., & Epstein, J. H. An ecological and conservation perspective on advances in the applied virology of zoonoses. *Viruses*, 2011, 3(4), 379-397. https://doi.org/10.3390/v3040379

160. Vicente, J., Montoro, V., & Vercauteren, K. C. Natural and Historical Overview of the Animal Wildlife-Livestock Interface. In Diseases at the Wildlife-Livestock Interface: Research and Perspectives in a Changing World. *Cham: Springer International Publishing*, 2021, 33-89. https://doi.org/10.1007/978-3-030-65365-1_2

161. Vijgen L.; Moës E.; Keyaerts E.; Li S.; Van Ranst M. A. Pancoronavirus RT-PCR Assay for Detection of All Known Coronaviruses. In: Cavanagh D. (eds) SARS- and Other Coronaviruses. Methods in Molecular Biology (Methods and Protocols). *Humana Press*, Totowa, NJ, 2008, 454, 3-12. https://doi.org/10.1007/978-1-59745-181-91

162. Wacharapluesadee, S.; Tan, C. W.; Maneeorn, P.; Duengkae, P.; Zhu, F.; Joyjinda, Y.; ... & Wang, L. F. Evidence for SARS-CoV-2 related coronaviruses circulating in bats and pangolins in Southeast Asia. *Nature communications*, 2021, 12.1, 1-9. https://doi.org/10.1038/s41467-021-21240

163. White, R. J., & Razgour, O. Emerging zoonotic diseases originating in mammals: a systematic review of effects of anthropogenic land-use change. *Mammal Review*, 2020, 50(4), 336-352. https://doi.org/10.1111/mam.12201

164. Wiederkehr, M. A., Qi, W., Schoenbaechler, K., Fraefel, C., & Kubacki, J. Virus Diversity, Abundance, and Evolution in Three Different Bat Colonies in Switzerland. *Viruses*, 2022, 14(9), 19-11. https://doi.org/10.3390/v14091911

165. Wilkinson, G. S.; & Brunet-Rossinni, A. K. Methods for age estimation and the study of senescence in bats. *Ecological and behavioral methods for the study of bats*, 2009, 315-325.

166. Wilson, A.G.; Lapen, D.R.; Mitchell, G.W.; Provencher, J.F.; Wilson, S. Interaction of Diet and Habitat Predicts Toxoplasma Gondii Infection Rates in Wild Birds at a Global Scale. *Global Ecology and Biogeography,* 2020, 29, 1189–1198. https://doi.org/10.1111/geb.13096

167. Wong, A. C. P., Li, X., Lau, S. K. P., & Woo, P. C. Y. Global epidemiology of bat coronaviruses. *Viruses,* 2019, 11(2), 1–17. https://doi.org/10.3390/v11020174

168. Wong, S., Lau, S., Woo, P., & Yuen, K. Y. Bats as a continuing source of emerging infections in humans. *Reviews in medical virology*, 2007, 17(2), 67-91. https://doi.org/10.1002/rmv.520

169. Wood, J. L., Leach, M., Waldman, L., MacGregor, H., Fooks, A. R., Jones, K. E., ... & Cunningham, A. A. A framework for the study of zoonotic disease emergence and its drivers: spillover of bat pathogens as a case study. *Philosophical Transactions of the Royal Society B: Biological Sciences,* 2012, 367(1604), 2881-2892. https://doi.org/10.1098/rstb.2012.0228

170. World Health Organization. Research priorities for the environment, agriculture and infectious diseases of poverty: technical report of the TDR Thematic Reference Group on Environment, Agriculture and Infectious Diseases of Poverty, 2013.

171. Xiao, J.; Prandovszky, E.; Kannan, G.; Pletnikov, M. V.; Dickerson, F.; Severance, E.G.; Yolken, R.H. Toxoplasma Gondii: Biological Parameters of the Connection to Schizophrenia. *Schizophrenia Bulletin* 2018, 44, 983–992. https://doi.org/10.1093/schbul/sby082

172. Yuen, K. S.; Ye, Z. W.; Fung, S. Y.; Chan, C. P.; & Jin, D. Y. SARS-CoV-2 and COVID-19: The most important research questions. *Cell & bioscience*, 2020, 10.1, 1-5. https://doi.org/10.1186/s13578-020-00404-4

173. Zhai, P.; Ding, Y.; Wu, X.; Long, J.; Zhong, Y.; & Li, Y. The epidemiology, diagnosis, and treatment of COVID-19. *International journal of antimicrobial agents*, 2020, 55(5), 105955. https://doi.org/10.1016/j.ijantimicag.2020.105955

174. Zhu, S., Shapiro, K., & VanWormer, E. Dynamics and epidemiology of Toxoplasma gondii oocyst shedding in domestic and wild felids. *Transboundary and Emerging Diseases*, 2022, 69(5), 2412-2423. https://doi.org/10.1111/tbed.14197