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Biodiversity loss and COVID-19 pandemic: The role of bats in the origin and the spreading of the disease

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ABSTRACT

The loss of biodiversity in the ecosystems has created the general conditions that have favored and, in fact, made possible, the insurgence of the COVID-19 pandemic. A lot of factors have contributed to it: deforestation, changes in forest habitats, poorly regulated agricultural surfaces, mismanaged urban growth. They have altered the composition of wildlife communities, greatly increased the contacts of humans with wildlife, and altered niches that harbor pathogens, increasing their chances to come in contact with humans. Among the wildlife, bats have adapted easily to anthropized environments such as houses, barns, cultivated fields, orchards, where they found the suitable ecosystem to prosper. Bats are major hosts for αCoV and βCoVs: evolution has shaped their peculiar physiology and their immune system in a way that makes them resistant to viral pathogens that would instead successfully attack other species, including humans. In time, the coronaviruses that bats host as reservoirs have undergone recombination and other modifications that have increased their ability for inter-species transmission: one modification of particular importance has been the development of the ability to use ACE2 as a receptor in host cells. This particular development in CoVs has been responsible for the serious outbreaks in the last two decades, and for the present COVID-19 pandemic.

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1. HOW biodiversity loss affects epidemics insurgence

The global impact of COVID-19 has reached more than 200 countries causing over a million of deaths (data of September 2020). Bats have been identified as the reservoirs of the current coronavirus, and it is believed that the spillover of the virus to humans was caused by the consumption of these Chiroptera. However, the current global disease emergence could have been the result of a convergence of three factors: (1) the diversity of wildlife microbes in a region (the zoonotic pool) [1]; (2) the effects of environmental changes on the prevalence of pathogens in wild populations; and, (3) the frequency of human and domestic animals contacts with wildlife reservoirs of potential zoonoses [2]. In general, biodiversity has been linked to reduced pathogen transmission through the so called dilution effect, which occurs when in a niche there is a variety of host species that negatively affect pathogen persistence by acting as buffering species [3,4]. However, the dilution effect does not appear to be universal, but depends on the animal community composition, host and pathogen ecologies, and the scale at which the system is examined [5–7]. For example, the biodiversity’ dilution effect will occur if increased species diversity leads to decreased host population density, that will reduce the contact rates, and therefore the transmission rate (β) (Fig. 1). Conversely, an amplification effect could occur if increased diversity leads to increased host density, with higher contact rates, and/or transmissibility [3,6–10] (Fig. 1).

Disruptive, extrinsic pressures in regions of high zoonotic potential where host or pathogen richness is already particularly high could trigger spillover to human hosts [11–14]. For example, the last 40 years have been characterized by a great decline of the environment in Southeast Asia (SEA), which has lost approximately 30% of its forest surface mainly caused by increased agricultural exploitation, and poorly managed urban growth [25]. The demographic growth in SEA has generated a great pressure on land use, with the most common activity being farming, logging, and hunting [15]. Anthropogenic pressure causes fragmentation of forest habitats,
anthropized environments, bats can easily adapt to anthropized rural and urban environments. In these intensification systems where biological and ecological processes are concentrated and are called ecotones - with the formation of patches, where the interfaces between them have been documented for several zoonoses such as Nipah virus encephalitis, influenza, rabies, hantavirus pulmonary syndrome [19,21–23].

Bat species are among the most numerous wildlife mammals that easily adapt to anthropized rural and urban environments. In these anthropized environments, bats can find niches compatible with their roosting and hunting needs: house lights attract a large number of insects at night, offering easy prey for insectivorous bats, while houses and barns offer shelter for cave-dwelling bats, while orchards and fields attract frugivorous bats [24–27]. These changes of the structure of the wildlife communities are characterized by low biodiversity, with an increase of the synanthropic species (a wild animal that lives in close contact with humans) population density that can elevate the contact rates, and thus increase the risk of pathogen transmission to humans [28–31]. This attractive effect of anthropized environments on bats with differing biological needs results in a higher concentration of bat-borne viruses, which increases the risk of transmission of viruses through direct contact with urine and feces, or through domestic animal infection [24–27,32–34]. The crowding and co-mingling of different host species will increase the number of pathogen genomes which will favor the production of strains that are more virulent and more adaptable to the new hosts through mutation, and recombination mechanisms [25,35].

Evidence suggests that many pathogens are transmitted between their animal reservoirs and humans but fail to be transmitted from human to human, or do so at rates that do not allow pathogen establishment within the human population. This condition has been termed viral chatters a seemingly common phenomenon of repeated transmission of viruses to humans, most of which fail to develop to a human-to-human transmission [36]. High rates of viral chatters will increase the diversity of viruses, and sequence variants moving into humans, raising the probability of transmission of a pathogen that can successfully replicate, and ultimately adapt to human environment [36–38]. This could be the way in which COVID-19 has emerged.

Bats are considered to play a key role in the evolution of coronaviruses, being the major reservoirs of alphacoronaviruses (αCoV) and betacoronaviruses (βCoV) [39,40]. Bats have been originated 64 million years ago, a time span that might have allowed the development of co-evolutionary processes between these Chiroptera and different virus species [25,41]. This phenomenon might have also favored the development of a strong correlation between bat ecology and coronaviruses diversity [25,42]. Moreover, the long co-evolutionary processes between bats and coronaviruses might have also contributed to the constitution of a vast pool of viruses with high opportunities of cross-species mixing, that has favored the development of strains with the ability to infect a vast range of hosts [43,44,62].

2. WHY are bat suitable reservoirs of coronaviruses?

Bats are members of the order Chiroptera and are the only mammals capable of sustained flight. There are approximately 1,230 species of bats, making these animals the second most numerous mammals after the rodents [45–47]. Bats fulfill many ecological roles from that of pollinators of fruit-bearing trees, playing a role in the processes of reforestation, to that of significant predators of insects, in particular crop pests and nuisance insect species [45]. Bats host more zoonotic viruses and more total viruses per species than rodents, and significantly higher proportion of zoonotic viruses than all other mammalian orders [44]. These viruses include, but are not limited to, various species of the genus Lyssavirus, henipaviruses, coronaviruses (e.g. SARS-CoV, MERS-CoV, COVID-19), and filoviruses such as several species of the Ebola virus [48–53].

2.1. Bat adaptation to viruses

Bats are the only flying mammals and this function has been accompanied by genetic changes to their immune system as a consequence of their metabolic rate. For example, bats produce large amounts of reactive oxygen species (ROS), and in response, have modulated genes to limit oxidative stress [54], which may result in reduced viral replication and pathogenesis [55]. Bats have high metabolic rate, compared to other animals. For example, metabolic rate in a running rat is 7-fold, and in a flying bird is 2-fold, while in bat
can reach 15/16-fold [56]. Filoviruses such as Marburg and Ebola can replicate at the bat flight temperatures of 37–41°C, indicating that the elevation in temperature does not affect their replication [56]. On the other hand, bats can also display a daily torpor with a decrease of the body temperature which might be a strategy to interfere with the optimal virus replication [56]. Moreover, bats display a unique interferon system (IFN) that might explain their ability to coexist with pathogens [57]. In general, mammals possess a large IFN locus that comprises a family of IFN-α genes expressed only following an infection. Conversely, in bats only three IFN-α genes are expressed, but constantly and constitutively [57]. This could be a highly effective system for controlling viral replication, which helps explaining bats’ resistance to viruses. In addition, bats lack the known PYHIN [PYRIN and HIN domain-containing] genes within the inflammasome pathway [54,58]. Natural killer immunoglobulin-like receptors (KIRs) are absent or significantly reduced in some surveyed bat species, potentially limiting disease and damage following infection [54,58]. Moreover, bats have very limited bone marrow or lack it at all, with the consequence of a low or absent production of B-cells, making bats asymptomatic carriers [54,58]. Another characteristic is their commensal relationship with the viruses [59]. The pool of viruses identified in the bats’ enteric samples may help their microbiome to enhance immunity [60]. All these factors can work in combination, and they can explain how, for example, diverse pools of CoV quasi-species can survive in bat populations [61].

2.2. Virus genetic variability in bats

The aspects that permit virus persistence in bats also contribute to their diversity and to the potential emergence of new viral species. As a result of flight for short periods of time, bats can accumulate ROS species which may have mutagenic effects. In the case of coronaviruses, the accumulation of ROS species could potentially overwhelm proofreading repair and/or altering viral polymerase fidelity, leading to virus species diversity, and therefore to cross-species transmission [62]. The constitutive expression of the IFN in bats may favor the selection of viral mutation that possess enhanced resistance to the antiviral defense pathways, providing a replication advantage after cross species transmission [57]. On the other hand, the absence of key inflammatory mediators in bat species provides no selective pressure to minimize these responses [58], which will favor a massive and pathogenic inflammation response in a new host as seen with both SARS-CoV, MERS-CoV [63,64], and COVID-19 infections in humans. In addition, virus recombination in bats are also common. The recombination frequency of coronaviruses, which can be as high as 25% for the entire genome [65], could make bats important reservoirs for coronavirus recombination and virus evolution, much like birds and pigs are for influenza viruses. Furthermore, the majority of recombination events identified in coronaviruses isolated from bats suggest recombination hot-spots around the spike gene [66,67].

2.3. Virus maintenance in a bat colony

Social organization in bats contributes to the maintenance of virus in the population. Bats are gregarious animals, living in colonies composed by thousands of individuals that often belong to more than one species [25]. For example, cave dwelling bats are characterized by multi-species association which shows higher frequency during maternity periods [25,68]. The transmission of the viruses in the bats’ colonies can occur through different ways depending on the bat and virus species considered i.e. aerosols, contact with feces, urine, blood, or other body fluids, or by bite [25,69].

Factors that can cause viral fluctuations within a bat colony can be the presence of high number of susceptible individuals, and stressful events. The inflow of susceptible bats into the colonies occur with the new births, or with the immigration of naïve animals from neighboring colonies, or with the expiration of immunity in previously infected individuals. These events can cause virus spreading within the colony and therefore keep the infection at high rates [69–71].

Stress-induced immunosuppression events in the bat population can increase susceptibility for contracting and shedding viruses. For example, a recent study demonstrated that secondary infection with the white-nose syndrome fungus (Pseudo-gymnoascus destructans) in bats increases CoV replication [72]. Weather conditions can also influence virus shedding and therefore possible spillovers. Inclement weather such as low precipitation can act as stressors, especially when associated with lack of food resources that can impair the immune system of the bats, causing increased susceptibility to pathogenic agents [73,74]. In fact, during rainy season roost sites may be limited for foliage-roosting bats, increasing the contact between individuals and thus more opportunities for pathogen transmission [73,74]. Therefore, seasonality might be an important driver of viral infections, including extreme weather conditions such as El Niño [75]. Precisely, the emergence of Nipah virus was also driven by the severe weather condition caused by El Niño. During dry season, when food resources are limited, bats are in poor body condition and might be less immunocompetent. Thus, individuals may become more susceptible to acquire viral infections in the subsequent rainy season [75]. For example, the year 2019 was characterized by El Niño, which even though was considered mild in comparison with previous events, it might have affected the resurgence of COVID-19.

2.4. Distribution of bat coronaviruses

The high diversity of bat species provides a great variety of cell types and receptors. Bats are found to be hosts of at least 30 different CoVs with complete genome sequences [76–87], and many more if those without genome sequences are included [88–97]. Bat coronaviruses are classified into four genera, Alphacoronavirus, Betacoronavirus, Gammacoronavirus (vCoVs) and Deltacoronavirus (dCoVs). Within Betacoronavirus, they can be further subclassified into lineages A, B, C and D [98]. In 2018, these four lineages were reclassified as subgenera of Betacoronavirus, and renamed as Embecovirus (previous lineage A), Sarbecovirus (previous lineage B), Merbecovirus (previous lineage C) and Nobecovirus (previous lineage D) [99]. In addition, a fifth subgenus, Hibecovirus was also included [99] (Fig. 2).

Among the four genera, only sCoVs and βCoVs have been found in bats, leading to the conclusion that bat CoVs are the ancestors for sCoVs and βCoVs, whereas bird CoVs are the ancestors for γCoVs and δCoVs [100]. Interestingly, for the jCoVs only those from subgenera Sarbecovirus (SARS-related CoVs), Merbecovirus (Ty-BatCoV HKU4, Pi-BatCoV HKU5, Hn-BatCoV HKU25, MERS-related CoVs), Nobecovirus (Ro-BatCoV HKU9 and Ro-BatCoV GCCDC1) and Hibecovirus (Bat Hn-betaCoV Zhejiang 2013) have been detected in bats so far [78,81,82,86,87,99,101], while several jCoVs from the subgenus Embecovirus (Murine CoV and CHoRCoV HKU24) have also been discovered in rodents [78,99].

Both sCoVs and βCoVs have higher detection rates in bats’ intestinal and fecal samples, making these animals’ excretions the major environmental source for the shedding of CoVs in spillover events [76,102–105]. For example, the conditions encountered in a wet market would be favorable to the infection of new hosts. In these places, animals of various species, including live bats, are kept in cages stored one above the other, with continuous contamination of food and water with excretions. The presence of a variety of animal species is also characterized by a great availability of
Fig. 2. Geographic distribution of bat CoVs from the genus Betacoronavirus. **A**: light green regions represent the countries with the presence of bat CoV from Sarbecovirus. **B**: orange regions represent the countries with the presence of bat CoV from Merbecovirus. **C**: purple regions represent the countries with the presence of bat CoV from Nobecovirus (modified from Wong et al. [181]).
different cell receptors that viruses can use to enter in a new host.

In general, αCoVs and βCoVs have been detected in bats in Asia, Europe, Africa, North and South America and Australasia [76,77,83,92,97,104–112], with αCoVs being more widespread and with higher detection rate compared to βCoVs (Fig. 3).

SARS-like-CoVs are present in different bat species but all belong to the family Rhinolophidae and Hipposideridae [101]. In China, horseshoe bat species (Rhinolophus spp.) are widely distributed, and are also the most frequent SARS-like-CoV carriers throughout the nation [66,76,101,102,113–123]. In fact, the largest population of completely sequenced bat’s SARS-like-CoVs were found in Chinese horseshoe bats (Rhinolophus sinicus) [66,76,111,123] (Fig. 4). It is also interesting to note that geographic factors also contribute to the diversity of SARS-like-CoVs. Precisely, the majority of SARS-like-CoVs are found in Yunnan province (China), and they share higher nucleotide identity (90–95%) with human SARS-CoV, while those found in southeast China, Korea and Europe shared only 77–90% genome nucleotide identity [66,115,124,125]. A similar situation also occurred with COVID-19. In this case, a SARS-like-CoV, RaTG13, was identified in Rhinolophus affinis from Yunnan province, and it showed an identity of 96.2% with COVID-19 [126]. Yunnan province is an ecological niche where many different species of horseshoe bats (Rhinolophus spp.) reside, and it is the geographical region with the highest diversity of bat SARS-like-CoVs with many recombination events among the various strains [66,101,115]. It is interesting to note that all strains of SARS-like-CoV capable of using human ACE2 as receptors were found in Rhinolophus sinicus and affinis only from Yunnan province, while other SARS-like-CoVs that cannot use human ACE2 were distributed in multiple provinces including Hubei province where Wuhan is located [66,113,114,117]. In addition, the ORF8 protein, that is important for interspecies transmission in CoVs, was found in viruses that were carried by Rhinolophus ferrumequinum and sinicus only from Yunnan province [66,115].

Thus, it was suggested that SARS-CoV most likely originated from Yunnan Rhinolophus bats via recombination events among existing SARS-like-COVs. Could these types of events also happened for COVID-19? Could the virus have jumped to humans in remote areas of South China, and then spread by human-to-human infection to the rest of the population? This hypothesis would be compatible with the fact that most of the hunting and guano collection activities are located in south China, and Southeast Asia, with higher probability of multiple spillovers, and therefore viral chatters that might have lead to the emergence of the new virus.

Bats have also the ability to harbor more than one species of virus at the same time, which might allow the CoVs to incorporate genes from other viral families through recombination events [113,127]. Examples of the coexistence of more than one virus in the same bat are: bat CoV 1 and HKU8–CoV [113,127]; HKU2-CoVs (SADS-CoV, the virus that caused the outbreak in pigs in 2016 in South China), and SARSr-CoVs [76,128]; two or more distinct genotypes of HKU9-CoVs [76]; HKU9-CoVs and a new identified filovirus (Mengla virus) that is phylogenetically related to the Ebola and Marburg viruses [129,130].

2.5. The coronaviruses machinery for successful spillover and emergence

Fidelity and gene acquisition in CoVs allow these viruses to alter key viral factors to overcome species barriers without sacrificing the function of other important elements [131]. Coronavirus genomes are the largest known RNA genomes (26–32 kilobases), and still being able to maintain functional components that allow it to be viable. The COVID-19 contains 4 structural proteins [132–137] and 16 non structural proteins (nps) [132]. In order to allow the emergence of new CoVs, these proteins must provide sufficient changes to overcome species barrier, maintaining, at the same time, key viral functions [132–137]. Two of the structural proteins (E and M) form the envelope of the virus, one (the N-nucleocapsid protein) binds the genomic RNA of the virus, the fourth, the S-glycoprotein, is the trimERIC protein that forms the typical protrusions from the surface of the virus that have given to it the "corona" name. It is perhaps the most important structural protein, as its task is the recognition of the ACE2 receptor of the host cell, and its interaction with it, that are the necessary steps that promote the penetration of the virus into the host cell. Importantly, the actual penetration of the virus demands the "priming" of the S-protein by host cell proteases that cleave to S-protein into S1 and S2 segments, exposing a sequence in the latter that fuses it with the plasma membrane of the host cell [138]. Mutations at S1 level, and adaptations within the new hosts are critical factors to ensure the emergence of new CoVs and pathogenesis [138]. Once the COVID-19 is incorporated of host cell, its RNA genome is liberated into its cytoplasm and reaches the ribosomes of the endoplasmic reticulum, where its open reading frames are translated into two proteins that are cleaved by cell proteases to yield the 16 nps [132]. One of them (nps 12) is the RNA-dependent RNA polymerase that, with the assistance of nps 7 and nsp 8 will synthesize the new RNA of the infecting virus. The function of the invading virus has additional complexities: for instance, a recent study has found that accessory proteins can also be taken from other pathogens [86]. Variations in the accessory proteins can create variability in infection characteristics and severity across CoVs’ families [139,140]. For example, a novel CoV (Ro-BatCoV GDCDC1) was found to contain 30 protein that were omologues to those of a known reovirus gene. This suggests the possibility of recombination activities even betvven viral families [139,140]. Therefore, CoVs have the ability to acquire proteins that allow them to survive in natural hosts and emerge as new ones [139,140].

3. Tracing bat coronaviruses spillover hotspots

Nine months after the onset of the epidemic in Wuhan, the city is still considered the place where the virus originated, even though a phyl–epidemiological study carried out by the Centre of Integrative Conservation (Xishuangbanna Tropical Botanical Garden) indicated that the COVID-19 found at Huanan market (Wuhan) could have been imported from other places [141]. According to the initial findings, the cause of the emergence of the current epidemic is the consumption of bats. Bat hunting practice is widespread in South China, Southeast Asia, Africa, and some Pacific Islands, and targets at least 167 species of bats [142]. In addition, guano extraction from bat caves, or harvested in bat farms is also a widespread activity, and the product is used as fertilizer in several countries, including Thailand, Indonesia, Vietnam, Cambodia, Mexico, Cuba, and Jamaica [112]. Bat excretions have been found to present high levels of α-CoV and β-CoV, and therefore guano extraction must be considered a concern for public health [76,102,103,105,112,143], and a risk for virus spillover [112,144].

In this chapter, we will review the practices of guano collection, bat hunting, and farming in Asia, and the related studies on coronavirus strains encountered in bats used for these activities.

Indonesia. Bat hunting is a very common practice in Indonesia which has caused the decimation of some bat species such as flying foxes (genus Pteropus and Acerodon). The bushmeat trade favors flying foxes because of their large body size and tendency to aggregate in large colonies, which increases the ease of capture [143]. In some regions smaller bat species were hunted because flying foxes were no longer present [145]. Bats are not considered an endangered species by the Indonesian Government, and the law allows the hunting and trading of these animals with a legal permit [146,147]. Before the bushmeat market reached its current form in Indonesia, local villagers
Fig. 3. Geographic distribution of αCoVs and βCoVs. A: pink regions represent the countries where αCoVs were discovered. B: blue regions indicate the countries where βCoVs were discovered. C: green regions represent the countries where both αCoVs and βCoVs were discovered (modified from Wong et al. [181]).
often hunted flying foxes for private consumption, or to sell them in traditional markets. Now bats are also available in some modern supermarkets in the cities [148]. In general, all the bats that reach the markets are already dead, but some places sell live individuals [148]. It is estimated that approximately 45–75 flying foxes are sold each day, with an increase to 150–450 on weekends [151]. The period of highest sale intensity if that of Christmas and New Year festivities, when approximately 1000 flying foxes are sold [148]. Bat are kept in cages with other wild animals such as endemic black macaques (Macaca nigra), pythons from Kalimantan (Python reticulatus or P. curtus) [149], water monitors (Varanus salvator), Sulawesi wild pigs (Sus celebensis), and Bornean bearded pigs (Sus barbatus) [150,151]. These conditions can exacerbate the spillover of viruses from bats to other animals facilitating the transmission to humans. In particular, the presence of Old World monkeys such as macaque in association with bats could potentially facilitate the transmission of CoVs from the primates to humans, considering the genetic similarities between the two species [152].

Vietnam. A survey performed by Huong et al. [153] from 2009 to 2014 assessed coronavirus spillover risk from different wildlife supply chains (rat and bat farms, and other wildlife animal farms) destined for human consumption [154]. In addition, guano farms were also sampled to assess the possibility of spillovers considering that bat artificial roosts are erected in gardens or backyards of wildlife farms with the presence of other farm animals such as duck, goat, chicken, and pigs [154]. The survey included 6006 registered wildlife farms across 12 provinces with approximately one million wild animals including rodents, primates, civet cats, wild boar, Oriental rat-snakes, deer, crocodiles, and softshell turtles. Ninety-five percent of the farms held 1–2 species of wildlife, and 70% of them also raised domestic animals on the same premises [154]. These farms sell wild meat to urban restaurants of all the country, and also supply international markets [155]. Commercial wildlife farming in Viet Nam is part of the expanded international trade of wildlife that is thought to have contributed to global epidemics, such as SARS and now COVID-19 [153,156–158].

Six distinct taxonomic units of coronaviruses corresponding to bat coronavirus 512/2005, Longguan aa coronavirus, avian infectious bronchitis virus (IBV), murine coronavirus, PREDICT_CoV-17, and PREDICT_CoV-35 were detected from the study of Huong et al. [153]. Phylogenetic analysis showed that among the six coronaviruses detected, PREDICT_CoV-35 and bat CoV 512/2005 clustered within the Alphacoronaviruses, while PREDICT_CoV-17, Longguan aa CoV, and murine CoV clustered within the Betacoronaviruses. The virus identified within the Gammacoronavirus genus was avian IBV [153]. Both PREDICT_CoV-17 and PREDICT_CoV-35 were detected previously in bats in Viet Nam, Cambodia, and Nepal, which confirms that coronaviruses are capable of infecting distantly related hosts [41]. The finding of the same virus in different bat species raises the question of whether they co-roost and/or share viruses through contact during other activities. Moreover, the presence of the same virus in bat species in multiple neighboring countries supports the hypothesis that viruses distribution is correlated with their bat hosts distribution [41,124,144] (Fig. 4).

Bat coronavirus 512/2005 was frequently found in co-infection with PREDICT_CoV-35 [153], while three types of coronaviruses (Longguan aa CoV, murine CoV, and IBV) were found in rats. The presence of a bat CoV and a poultry CoV in rats can be explained by the presence of multiple species on the wildlife farms, which may facilitate viral recombination leading to the emergence of new viruses [66,127]. The transit of multiple animal species through the supply chain offers opportunities for inter- and intra-species mixing. Animals confined in overcrowded cages with poor nutrition can increase stress which contribute to shedding of coronavirus and amplification of the infection through the supply chain for human consumption, including close direct contact with traders, butchers, cooks, and consumers [159,160].

Moreover, a high proportion of bat feces were found positive to coronaviruses at bat guano farms, indicating the potential risk of bat guano farmers, their families, and their animals being exposed to the viruses [66,127].

Cambodia and Lao PDR. In Cambodia and Laos several thousand bats per day are sold in the markets [161]. Bat are also exported from these countries to markets in Thailand [162]. In Cambodia and Laos, small farmers also practice artificial bat roosting in their backyard farms for guano collection, with other domestic animals sharing the same environment. A study conducted by Lacroix et al. [163] found a significant diversity of CoVs in bats both in Cambodia and Laos. For example, it was reported that Pipistrellus bats hosted both αCoV and βCoV, a finding that raises concern since this species is very common in rural and urban areas [80,164–167]. In addition, the health risk in Cambodia is worsened by the fact that Pipistrellus bats are also hunted for food, beside being used for guano extraction [168,167]. Furthermore, hunters, restaurant workers, and farmers have been reported to be bitten by bats, and to have been exposed to their urine and feces. Farmers, who wear no protective equipment or clothing, collect guano in tarpaulins or nets laid beneath the bats’ roosts, and are regularly urinated on. The exposure of the farmers to the bat guano by direct contact with urine and feces must therefore be considered [163]. In fact, a study conducted by Wacharapluesadee et al. [111] identified HKU1 betacoronavirus in a non-hill individual with high level of occupational exposure to guano.

Thailand. Bats are hunted for consumption also in Thailand. They can be easily found in restaurants in Bangkok, and in the daily markets in north-east regions of the country [143,162]. Some bats may be transported from Laos, indicating cross-border trades. Bats play a cultural role in Thailand, including medicinal, religious, and culinary functions [168]. A survey conducted by Suwannarong et al. [169] reported the highest bat consumption in the Northern region of Thailand (Chiang Mai and Chiang Rai at the border with Burma and Laos). Surveyed people responded that they had consumed bats at least one time in the previous 6 months. Bats were mostly obtained from caves, purchased in local markets, or hunted by locals [169].

Philippines. In Philippines over 1500 bat caves have been recorded. Most hunters kill only a few bats per trip, but those operating at roost sites take hundreds in a single hunt [170]. In the country, bats are mostly hunted for food, but bats’ caves are also frequently visited for guano extraction [171,172]. Locals and some indigenous groups usually hunt larger species of bats such as fruit and large hippocephalids bats for protein source and trade. A clear indication of bat hunting and exploitation in caves is the presence of carcasses and bones of bats, and coconut torches that may have been used to smoke the caves [143].

China. The bat meat trade is mainly found in the Southern China, where bat can be traded locally and regionally. Some restaurants in Guangdong and Guanxi provinces offer bat dishes on the menu [143]. Bats are not a protected species in China, and during the last 30 years the population of these animals has shown a 60% decline, mainly caused by bat cave exploitation for tourism, and poaching by locals [173]. Surveys carried out in Guangdong province between 2005 and 2012 revealed the consumption of bats by the majority of the respondents [174,175]. Live bats were found to be sold at wet-markets in Guangzhou and Gaozhou (Guangdong province) [174]. In addition, cases of dead bats found in oyster sauce containers were reported by customers. The oyster sauce was produced by a factory in Guangdong province. The presence of the bats in the processed food was incidental, probably caused by the animals roosting in the production areas, and falling inside the containers [176]. Whole bats, body parts, or desiccated feces are also used in the Chinese Traditional Medicine, which raise great health concerns [177].
3.1. Malaysia and Nepal

Bats are regarded as luxury meat in Malaysia with most vendors selling 200–300 bats per season [178]. In Nepal, bats are also used as food by the local communities [143].

3.2. A general point

A final comment is in order at the end of this contribution: bats are kept in the markets with other animal species, which could amplify the spillover events. Among the animals sold at the markets, there are Old World monkeys such as macaques. In a recent study Damas et al. [179] have examined the ACE2 receptors from 410 vertebrates (fishes, amphibians, birds, reptiles, and mammals) to predict their ability to bind COVID-19 spike proteins. The ability of the vertebrates was classified as low, medium, and high. The 18 species classified as “very high” were all Old World monkeys, whose S-protein binding residues in the ACE2 receptors were identical to those of humans. Among these primates, Rhesus macaque (Macaca mulatta) is the only known species infected with COVID-19, and showing symptoms similar to those in humans [179]. Macaques of the species Macaca fascicularis and Macaca nigra are the most common primate traded in Cambodia, Malaysia, Philippines, and Indonesia to be sold in laboratories, and is also used for consumption in South China [152]. Therefore, an hypothesis for an intermediate host could be propose here. Even though different SARS-like-CoVs have the ability to use ACE2 as entry receptors, thus arguing against the necessity for them of an intermediate host to reach humans [113,120], macaques might be proposed as likely susceptible hosts for COVID-19. This hypothesis could be further supported by the fact that these primates are kept in markets in association with bats, raising serious concerns on the probable spillovers from these animals to humans.

4. Conclusions

Anthropogenic changes such as deforestation, habitat fragmentation, land-use, agricultural development, and uncontrolled urbanization can impact the transmission of infectious disease from animals to humans by altering the biodiversity’s dilution effect. These changes also alter pathogens’ niches, and the movements of hosts and vectors preparing the general ground that favors the emergence of infectious diseases. The pathogenic dynamics of CoVs only demand a host as the permanent reservoir, and albeit not necessarily, an intermediate host. In Southeast Asia, the permanent reservoir of CoVs are the bats, which in this region are commonly consumed as food. Evolution has shaped the metabolic properties of bats and their immune system in ways that have made them insensitive to the pathogenic effects of CoVs. The CoVs themselves have also undergone evolutionary changes that have increased their pathogenic potential: one of particular importance in the present pandemic, and in previous outbreaks (SARS-CoV), has been the ability to use the ACE2 receptors of target cells to invade them. The presence of multiple animals species in the markets in SEA and South China, including bats, produces a high density of possible susceptible hosts, which creates an Amplification Effect Zone increasing the probability of spillovers. Macaques are most frequently encountered primates in these markets, and are also raised in farms in different SEA Countries to be sold to laboratories, or for food consumption in particular in South China. Considering that macaques have a close phylogenetic relationship with humans [180], it might be easier for CoV to use them as intermediate hosts.
for the spillover of the virus to humans. Therefore, the possibility that macaques used in the wildlife trade chain could be intermediate hosts for COVID-19 should be given serious consideration.

Declaration of competing interest
I declare no conflict of interest.

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