HEALTH PROMOTION

Improving the ONE HEALTH approach: a lesson from SARS-CoV-2 pandemic

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Summary

The emergence of SARS-CoV-2 has underscored the critical need to enhance the ONE HEALTH approach which recognizes the interconnectedness of human, animal, and environmental health. In this review we report on various animal species that were infected by SARS-CoV-2 virus during the pandemic with the aim to contribute to the One Health approach. The SARS-CoV-2 pandemic has highlighted the devastating consequences of zoonotic diseases such as COVID19 and has reiterated the critical role

Introduction

SARS-CoV-2, a highly pathogenic coronavirus in humans, has emerged at the end of 2019 and has caused a devastating pandemic with 771,549,718 confirmed cases, including 6,974,473 deaths, as of 25 October 2023 [1]. Although its origin is still unclear, SARS-CoV-2 has been acknowledged to be a zoonotic virus, meaning that the virus can spread from animals to humans [2]. The most accepted hypothesis behind the COVID-19 pandemic is that the virus originated in a bat species and then spread to humans through a direct spillover event. Additional hypotheses are 1) the circulation of a less-virulent progenitor that over time accumulated mutations and increased its virulence or 2) the involvement of an intermediate host which, having been infected by a bat, was then able to transmit the virus to humans. Although the involvement of an intermediate host has not been confirmed, pangolins and civets have been identified as potential candidates [3-6] (Fig. 1).

As of September 2022, SARS-CoV-2 had been detected in 25 animal species (wild, captive, and domestic animals) from 36 countries. Most of these animals were infected after contact with sick humans, a phenomenon called reverse zoonosis [2, 5, 7, 8]. The main concern during reverse zoonosis is the possible increase of viral virulence due to the many mutations the virus may accumulate during spillover events. These mutations may result in immunity escape factors that when the virus will spread back to humans may lead to disease

that the health of wildlife, domestic animals, and the environment plays in human health. The pandemic is a lesson learnt on the urgent need for an enhanced ONE HEALTH approach by developing a robust and interconnected global health strategy to effectively prevent and control zoonotic diseases and protect the health of all species on our planet. These efforts are crucial for a sustainable and resilient future for both human and ecological systems.

worsening [5, 7]. The "One Health Concept", first used in 2003-2004 following the emergence of Severe Acute Respiratory Syndrome (SARS) in 2003, recognizes that human health is strictly related to the health of animals, plants, and their shared environment, focusing on the relationship and interconnections among these entities [9-12]. Short after the 2003 SARS epidemic, the emergence and spread of the highly pathogenic avian influenza virus H5N1 [13, 14] contributed to the growing importance of "One Health Concept". The European Commission strongly supports and encourages a One Health approach when it comes to the prevention, preparedness, and response to zoonoses, especially major threats such as avian influenza viruses, West Nile and Ebola viruses. Prevention, robust surveillance, rapid detection and response are crucial when dealing with serious cross-border health threats such as zoonoses [15]. The COVID-19 pandemic has further highlighted the need for an improved One Health Concept that aims to a collaborative, multi-sectoral, and transdisciplinary approach to address potential health threats from the animal-human-environment interface [7, 9, 10, 16-18]. Considering that about 61% of common human pathogens and about 75% of emerging pathogens are of zoonotic origin, mainly from wildlife, surveillance measures of domestic animals as dogs and cats, and of secondary wild reservoir species as mink and whitetailed deer [5] are critical for preventing the emergence of novel SARS-CoV-2 variants as well as of other emerging pathogens. For this reason, the relationship

between the environment and human and animal health is fundamental to understanding and addressing global health challenges [19]. For instance, pollution, climate change, and habitat destruction can lead to the emergence and spread of infectious diseases, affecting both wildlife populations and human communities [20]. Conversely, preserving biodiversity and maintaining healthy ecosystems can provide essential ecosystem services that support human health, such as clean air, water, and food security [21, 22].

The aim of this work was to provide an overview of the various animal species infected by SARS-CoV-2 in order to contribute to the One Health approach.

SARS-CoV-2 infection in pets

Domestic animals, also called pets, are all those which are kept at home for company, such as cats, dogs, ferrets, and hamsters. The COVID-19 pandemic greatly changed the pet-owner relationship. Indeed, while social isolation increased the adoption of pets [23], many pet owners abandoned their animals for fear that they could vehicle the virus [24]. In addition, preventive behavioral measures were taken to avoid direct contact with pets and as a consequence owners became less closely involved with their animals [25].

Experimental studes have shown that pets are susceptible to SARS-CoV-2 infection [26] (Fig. 2). However, their role in the virus transmission cycle is not yet clear.

The first cases of human-animal transmission occurred

in Hong Kong in February and March 2020, when two male dogs, one Pomeranian and one German Shepherd, tested positive for SARS-CoV-2 with RT-PCR and serological tests [27]. Serological studies have detected very low SARS-CoV-2 antibody titers in dogs and clinical signs are rarely present. While dogs are the animals living in close contact with humans, their seroprevalence rate is less than 5%, thought it tends to increase when their owners carry a high viral load [28, 29]. In dogs, sex and age seem to play a role in the risk of SARS-CoV-2 infection, and susceptibility to infection increases in male dogs older than year old [30].

Cats can transmit the virus by air to other naive cats causing infection in the lungs, lymph nodes enlargement, coughing and dyspnea [31]. Cat infection acquired from COVID-19 positive owners has been reported from various countries, confirming human-tocat SARS-CoV-2 transmission. In a study conducted in Hong Kong, 12% of cats living with infected humans tested positive for SARS-CoV-2 [32]. Similarly, a study conducted in Wuhan (China) found that 14.7% of cats living with infected owners tested positive for the virus. Of these, 10.8% had neutralizing antibodies against SARS-CoV-2 [33]. Cat-to-human transmission seem a rare event with only one case reported in Thailand, where an infected cat of an infected owner sneezed on a veterinarian who became infected [34]. Similar to humans, clinical signs as fever, lethargy, respiratory distress are also observed in cats but are not specific to COVID-19 infection. Younger than one-year cats have a higher risk of infection than dogs of the same age [35]. Cats are more susceptible to infection than dogs, probably because they retain high greater expression of Angiotensin-Converting Enzyme 2 (ACE-2) receptors, the target of the SARS-CoV-2 spike (S) protein in the respiratory tract [36]. Viral persistence in cats is longer (21 days) than in dogs (13 days) [37] and cats' genomic sequence encoding ACE-2 receptor shows 85.5% similarity to that of humans. Only four out of 20 amino acid residues encoding the receptor-binding domain (RBD) of the S protein have been found to differ between cats and humans [38].

Ferrets are susceptible to SARS-CoV-2 infection and have been shown to transmit the virus to other ferrets [39]. In several countries ferrets are kept as pets, but little information is available on SARS-CoV-2 epidemiology in these animals. In a study conducted in Spain in ferrets living with humans infected with SARS-CoV-2 it was found a seroprevalence of 1.6%. None of the seropositive animals showed clinical signs of SARS-CoV-2 infection [40].

Hamsters are considered to be a valuable animal model, as they can develop SARS-CoV-2 infection [41]. Golden Syrian hamsters inoculated nasally with SARS-CoV-2 developed several signs of infection and transmitted the virus to naïve co-housed hamsters [42, 43]. Viral transmission from humans to hamsters was reported after infected Golden Syrian hamsters were shipped from the Netherlands to Hong Kong and sold to different pet shops. The subsequent animal-to-human transmission

resulted in more than 80 human cases all of which were attributed to the Delta variant [44, 45].

SARS-CoV-2 in farm animals

SARS-CoV-2 infection has also been described in farm animals (Fig. 2).

Pigs are considered to have a limited susceptibility to SARS-CoV-2 [46]. Under experimental conditions they shown to be resistant to intranasal inoculation of SARS-CoV-2. Indeed, no clinical signs, including increased body temperatures, were observed in these animals [26, 47]. Low level of viral shedding and antibodies against SARS-CoV-2 were found only in 31.3% of experimentally infected pigs [48].

Poultry is also considered at low or no susceptibility to SARS-CoV-2 infection. In a study SARS-CoV-2 was inoculated into chickens, turkeys, ducks, quails and geese and no clinical signs were observed. The virus was not detected in any swab material, and antibodies were not detected in the serum of any species. These results suggest that either SARS-CoV-2 does not replicate in any of the studied poultry species, or that it replicates at too low a level to be detected [49]. Similar findings were reported from other studies performed in chicken [26, 47].

Cattle have little susceptibility to SARS-CoV-2 infection. In one study, as early as 3 days after experimental

infection, they displayed a low viral load with a mild but detectable serological response and no cattle-to-cattle transmission was occurred [50]. In another study cows and buffaloes tested negative to RT-PCR for SARS-CoV-2 after contact with their molecular swab-positive owner [51]. Occasionally cattle can be infected and seroconvert after contact with infected humans as found in another study [52]. In sheep, the replication of SARS-CoV-2 in respiratory tissues has been demonstrated [53, 54]. However, limited transmission to other sheep has been reported [53]. Another in vivo study on experimentally infected sheep found no virus or viral RNA in swabs or tissues, but some of them developed low titer of neutralizing antibodies, suggesting a mild infection [55]. A serological investigation on sheep in close contact with humans conducted before and after the pandemic did not find SARS-CoV-2 antibodies, suggesting that sheep are not or little susceptible to SARS-CoV-2 infection [56].

Goats have low susceptibility to SARS-CoV-2 infection. Experimental studies with intranasal inoculation found that animals became positive after 7 days with no changes in body temperature suggesting that goats may have very low susceptibility to SARS-CoV-2 infection [55, 57].

Rabbits are susceptible to SARS-CoV-2 [55, 58]. The infection, despite the positive swab, is asymptomatic, with a low viral replication suggesting that virus transmission among these animals may be less efficient

than in ferrets and hamsters; also, a low viral replication was observed. Finally, a study of more than 100 rabbits showed a very low seroprevalence rate [59]. The spread of the virus and disease in other rabbit breeds or in rabbits of different ages should be further investigated as no data are available [58].

An experimental infection of an adult horse by intranasally administered ancestral SARS-CoV-2 revealed no viral shedding [55]. Some studies involving the molecular detection of SARS-CoV-2 in nasal secretions and/or in feces from healthy horses that had been in contact with infected humans and sick horses revealed no evidence of virus spread [51, 60]. Moreover, a serological survey conducted in China on 18 healthy horses did not detect antibodies against SARS-CoV-2 [61]. However, a study of healthy racehorses found a seroprevalence of 5.9%, suggesting a possible spillover from SARS-CoV-2 infected humans to horses [60]. The seroconversion of a horse after close contact with infected humans confirmed the possible reverse zoonosis occurrence between humans and horses [62]. Finally, in a large seroprevalence study including 1,186 equines it was found that 3.5% of horses had detectable SARS-CoV-2 antibodies without showing clinical signs [63].

The low binding affinity to ACE-2 receptor studied by comparative sequence analysis and functional studies in equines (horses and donkeys) explains the low susceptibility of the species to SARS-CoV-2 infection [64, 65].

SARS-CoV-2 on mink farms

Minks were the first animal population that emerged as a potential source of spillback (the spreading of the virus from humans to animals, also called reverse zoonosis) and secondary spillover (transmission from new animal hosts instead of the original host) during COVID-19 pandemic. The initial infection of these animals was caused by infected humans working in mink farms. An intense and sustained animal-toanimal transmission followed as a result of both the high-density of mink populations and of the high susceptibility of minks to SARS-CoV-2 infection (Fig. 2). This intense intra-species transmission in minks led to the emergence of novel viral variants that spilled over into humans [66].

The first confirmed cases of SARS-CoV-2 in mink were reported in the south-east of the Netherlands during the first pandemic wave [67]. By the end of April 2020, mink from two different farms showed respiratory and gastrointestinal disorders, with a mortality rate of 1.2–2.4%. The infection rapidly spread throughout the farms, with many animals clinically affected. Histological signs of acute interstitial pneumonia and other lung lesions were observed, and viral RNA was detected in the lungs, throat, liver, and intestines of the dead animals [68]. Epidemiological investigations found that SARS-CoV-2 was initially transmitted to minks by humans and then spread widely among mink.

Approximately 68% of farm workers or their contacts were subsequently infected with SARS-CoV-2. Viral phylogenetic sequencing confirmed that humans were infected by mink viral strains that differ from the viral strains circulating among humans who had not been in contact with minks [69], providing evidence of minkto-human transmission [70].

Subsequently, several outbreaks of SARS-CoV-2 infection were reported from mink farms in Europe and North America resulting in high mortality and great economic loss for mink farming [69, 71-75].

A SARS-CoV-2 variant of mink origin, named 'Cluster 5', was initially observed in an outbreak in mink and humans in Denmark in June 2020 [76]. The identification of this mink-originating variant led to the mass culling of mink to minimize risks to of wider spreading to humans [77]. In fact, at that time approximately 53% of SARS-CoV-2 strains infecting humans in Denmark were of mink origin, indicating generalized secondary zoonotic transmission had occurred [66]. Outbreaks like those reported in Denmark have persisted throughout the pandemic with the isolation of mink viral strains identical to those isolated in humans [78] and with emergence of other variants of mink origin, such as the Marseille-4 variant (B.1.160) [79]. In addition, transmission from farmed mink to semi-domesticated animals has been documented in the Netherlands [80], and infection from wild minks has been reported in Spain and USA [81, 82], fueling concern about the prospect that a highly susceptible wild animal can become a SARS-CoV-2 reservoir.

Possibly on account of host adaptation, the virus appeared to evolve at a faster rate in mink than in humans [70]. Whole genome sequencing technique has identified about 170 mutations in SARS-CoV-2 samples collected from mink farms, some of which have been found also in humans [83]. The good adaptation of the virus to the mink host led to the emergence of variants, with accumulation over time of point mutations that were repaired early during the spread of the virus in minks enabling the virus to be easily reintroduced into humans [71, 84]. Rabalski et al. [84] found four distinct mutations in the S gene in an isolate taken from a farm worker who tested positive for SARS-CoV-2. This viral variant gave origin to the mink-adapted variant. The detection of mutations in the RBD of the S protein raises concerns about the efficacy of vaccines against human infection by S protein variant strains [72].

SARS-CoV-2 in wild animals

Some studies have reported SARS-CoV-2 spillover from human to wild animals [85] (Fig. 2). The infected wild animal species were primarily in contact with humans in settings of captivity, such as zoos, safari parks, zoological centers, and aquariums [86]. SARS-CoV-2 has been found in 17 animal species belonging to three orders: Artiodactyla, Carnivora, and Primates. Except for two species of the order of Artiodactyla, namely deer, and

one of the order of Primates, the other 14 cases concern species belonging to the order of Carnivora, family of Felidae, with the highest number of species in which the virus has been found [87].

The first case of animal infection by SARS-CoV-2 in the USA was reported in a Malaysian tiger at the Bronx Zoo, New York, as the first case of viral transmission from human to a non-domestic animal [3]. The tiger appears to have been infected by an asymptomatic zookeeper who tested positive for SARS-CoV-2 [88]. Subsequently, the infection was detected in other four tigers and in lions, confirming that SARS-CoV-2 can be transmitted to various species of felines [3].

The emergence of the Delta variant (B.1.617.2), first identified in India in October 2020, prompted the assessment of its possible circulation in wild animals, such as lions [89]. In May 2021 the occurrence of natural infection by the Delta variant was reported in Asian lions, in the Arignar Anna Zoological Park in Chennai (India), where four of thirteen lions had symptoms of SARS-CoV-2 infection, such as loss of appetite and cough. A total of 11 lions were then tested using RT-PCR, nine were positive for SARS-CoV-2 and two of the positive animals died. Although it appeared that the primary source of infection was an infected person, with the further transmission of the virus occurred for the close contact among animals that share the same habitat [90]. The Delta variant was also found in another two cases of infection in Asian lions [89].

Cases of natural infection in pumas occurred in a private zoo in Johannesburg, South Africa, during the first and second epidemic wave. The first positive case of SARS-CoV-2 was found in a puma with symptoms of anorexia. The second case occurred 24 hours later in another puma with similar symptoms. An investigation on zoo staff was performed and two individuals had positive swab [91].

The first two cases of SARS-CoV-2 infection in snow leopards occurred at Louisville Zoo (Kentucky, USA) in December 2020. Again, the infection appears to have been caused by a zookeeper who tested positive to SARS-CoV-2. Later, SARS-CoV-2 infection cases in snow leopards were reported in California, Nebraska, South Dakota, and Illinois due to human-to-animal transmission [87].

In 2021, the transmission of SARS-CoV-2 from man to white-tailed wild deer (Odocoileus virginianus) was documented in the USA [92]. In July 2021, antibodies against SARS-CoV-2 were found in 152 whitetailed deer with a 40% seroprevalence in Michigan, Pennsylvania, Illinois, and New York. In another study between January and March 2021, SARS-CoV-2 was detected in 35.8% out of 360 swabs collected in nine locations in northeastern Ohio. Male and heavier deer were more likely to be infected, especially those living near urban centers where they were more likely to have direct contact with humans through garbage, backyard feeders, and bait stations [93].

In Switzerland, a case of SARS-CoV-2 infection was reported in a red fox during routine surveillance testing [94].

In another study, 283 samples of nasopharyngeal lymph nodes from wild and captive deers were collected in Iowa from April 2020 to January 2021. Of these, 33% were positive on RT-PCR. The majority of the samples were collected between September and December 2020, suggesting that spillover occurs mainly in the hunting season [92]. As more than a third of the deers tested positive to SARS-CoV-2, the authors alerted that a potential new reservoir of SARS-CoV-2 infection might arise.

Naturally acquired infection has also been ascertained in captive gorillas in USA, Czech and Spanish zoos, as a results of direct and indirect contact with SARS-CoV-2 infected humans. Great apes and Western Lowland gorillas displayed visible signs of infection, such as nasal discharge and coughing, and pneumonia-like signs were observed in old apes [66, 95].

Finally, in a zoo in Belgium, hippopotamuses displaying only nasal discharge were found to be swab-positive for SARS-CoV-2. The slight genetic difference between the human and the hippopotamus viruses suggests that humans had infected these animals [96]. Other cases of infection in captive animals have been reported in fishing cats, hyenas, lynxes, mandrills, monkeys, and otters [66].

Conclusions

This work provides an overview on SARS-CoV-2 infection in different animal species. Data were retrieved from both experimental studies and natural infection events occurred during the COVID-19 pandemic.

There is clear evidence that zoonotic spillovers and reverse zoonosis occurred during SARS-CoV-2 pandemic involving humans and animals, providing a comprehensive understanding of the virus transmission dynamics for supporting the improvement of One Health approach strategy.

One Health is a transdisciplinary approach to understanding and addressing complex challenges that involve the health of humans, animals, and the environment [14, 97]. It recognizes that the health of these three interconnected domains is closely linked and that health issues in one domain can have a significant impact on the others. This approach considers health within a unified global perspective (One Health) and it is supported by the evidence that the various outbreaks of new infectious diseases that have periodically occurred during the past five decades are due to zoonotic spillovers [11].

Many species, some of which are in close contact with humans, are susceptible to SARS-CoV-2. The susceptibility of species may change over time and viral variants may continue to emerge with changes in transmission capacity, potentially giving rise to the appearance of new hosts [98]. In the case of mink and deer infection, the adaptation of SARS-CoV-2 via only minimal mutations illustrates the capability of adaptation of this virus to new animal species. In the most susceptible species, the possibility that new viral mutations emerge will increase the risk of secondary spillover and reverse zoonosis transmission.

In this review we have reported that wild animals were infected by SARS-CoV-2. It is of concern the possibility that a wild animal can become a reservoir of SARS-CoV-2, as it would represent a new potential transmission route for further spread of the virus more difficult to control than the reservoir of domestic animals. An assessment of the current epidemiological situation like the one approached in this review, may serve to recommend options for reviewing the monitoring strategies for SARS-CoV-2 infection in animal species of concern.

In this context, the continuous surveillance of both humans and animals is essential as new variants of SARS-CoV-2 could emerge creating a dynamic landscape of susceptibility and transmission within and between species. Such development could have far-reaching implications for conservation, ecosystem health, food production, economics, and public health. Also, further research is needed to identify potential reservoirs of the virus and to further investigate the human-animal transmission route. Finally, all those animals that are susceptible to the virus, whether domestic, intensively farmed, or wild in captivity, should be vaccinated in order to limit the development of overly diffusive or pathogenic SARS-CoV-2 variants [86, 99]. This approach can help limit the development of overly diffusive or pathogenic SARS-CoV-2 variants. Additionally, enhancing biosecurity measures in animal farming and wildlife management can reduce the risk of spillover events [100, 101]. However, in Europe, vaccination of animals against SARS-CoV-2 was only applied in mink in Finland but is no longer in use due to the limited effectiveness against Omicron variant. Data regarding other possible impact of the vaccination, such as the spread of the virus, the efficacy versus infection and the onset or duration of immunity were incomplete to allow a reliable assessment. Overall, it seems that the vaccine was able to provide a certain degree of protection against severe disease caused by the Delta variant in mink, but it did not prevent infection [102]. Moreover, promoting sustainable agricultural practices can also minimize human-wildlife contact, thereby reducing the risk of zoonotic disease transmission [103].

Overall, the information coming from monitoring programs in animal populations can aid the assessment of risks of virus transmission from animals to humans, with particular regard to the possible selection and emergence of new variants in animal populations, for which humans might be more susceptible or available vaccines less effective [102].

Control of the SARS-CoV-2 pandemic required the adoption of One Health as a collaborative global approach to mitigating the risk to both humans and animals and to uncovering and attenuating the severity of a complex human-animal environmental health problem. However, there road is still long to achieving an optimal institutional coordination and collaboration across various disciplines, a pathway that necessitates the

inclusion of social, cultural, and economic components to create a solid One Health approach [104, 105]. Furthermore, establishing interdisciplinary teams that include veterinarians, ecologists, public health professionals, and social scientists can enhance the effectiveness of disease prevention and control strategies [106].

As already reported for Monkeypox virus [107], the implementation of the concept of One Health in all human, animal and environmental spheres could contribute substantially to both to the control of new outbreaks and to strengthen public health preparedness and response.

Finally, educating communities about the importance of One Health and promoting behaviors that reduce the risk of zoonotic transmission can support long-term public health resilience [108, 109].

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Ethics approval

Not required.

Conflicts of interest

Emanuele Montomoli is founder and Chief Scientific Officer of VisMederi srl and VisMederi Research srl. Claudia Maria Trombetta is an external consultant of VisMederi Research srl. The remaining authors have no conflicts of interest to declare.

Authors' contribution

Conceptualization: CMT; Writing - original draft preparation: SM, GG, MGM, BP, CMT; Writing - review and editing: SM, GG, MGM, BP, SV, GM, EM, VM, MC, CMT; Visualization: SM; Supervision: CMT; Project administration: SM, GG. All authors approved the final version of the manuscript. SM and GG contributed equally to this work and share the authorship.

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