Hayvanlarda SARS-CoV-2 enfeksiyonu

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Abstract

In December 2019, a new respiratory illness was identified in human in Wuhan and has spread across the world. Causative agent of the novel coronavirus (COVID-19) outbreak soon named as SARS-CoV-2. After World Health Organization has declared global emergency in March 2020, the disease has raised dramatically and over 32.7 million COVID-19 infected cases with 991 000 deaths has been reported until late September 2020. The role of animals either as reservoir or target host has been intensively discussed. It has been suggested that bats may be a possible genetic source in the onset of the disease. However, the determination of the possible intermediate host that may have a role in the transmission of the disease to humans is still exactly unclear. In addition, considering the mutation possibilities for the virus with such a high transmission rate, an increase in the host range or surpassing the interspecies barrier is also a situation that can be encountered. Though epidemiologic role of animals in SARS-CoV-2 outbreak must be monitored intensely. In parallel with the increasing disease rates, the information gained on COVID-19 infection is updated rapidly as well. In this review article, prospecting possible interactions between animals and COVID-19 outbreaks which highly affected human health is aimed.

Keywords: SARS-CoV-2, COVID-19, animal infection, zoonosis, reservoir hosts

Öz


Anahtar kelimeler: SARS-CoV-2, COVID-19, hayvan enfeksiyonu, zoonoz, rezervuar konakçı
Introduction

As of current knowledge, approximately 70% of pathogens that infect humans originate from animals. Because of high mutation rates due to the genetic recombinations in the positive-sense RNA viruses or reassortment between segmented genomes, those outbreaks mostly attributed to RNA viruses (Chan et al. 2013). There are environmental factors which affect the emergence of the viral diseases. In the transition of human communities from hunting to settled order, the ecology has been affected and contact with various wild animal species has increased. This situation allowed viruses to access into different hosts. Today, the acceleration of human travel has increased the virus spread and allowed it to reach isolated areas. As a result of cultural interaction, the demand for increasing the use of different animal species for food and games has increased the contact of people with animals that act as reservoirs for different viruses. Like in influenza viruses which interspecies transmission occur frequently, it is still unknown whether mammalian or avian species attributes to SARS-CoV-2 circulation. This review focuses on the role of animals at evaluation or to the course of COVID-19.

Coronavirus properties and tropism

Coronaviruses are enveloped viruses which consist of single-stranded, non-segmented RNA which composed of about 26-32 kb nucleotides (Wu et al. 2020). Alphacoronavirus, Betacoronavirus, Gammacoronavirus and Deltacoronavirus are the genera classified into the family Coronaviridae. SARS-CoV-2 belongs to the Sarbecovirus subgenus in Betacoronavirus genus which also includes the other viruses namely Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV). SARS-CoV-2 differs from SARS-CoV and MERS-CoV with a low genetic similarity of 79% and 50%, respectively) (Lu et al. 2020). Spike protein (S), membrane protein (M), envelope protein (E), and nucleocapsid protein (N) are the structural proteins encoded by SARS-CoV-2 genome (Kalita et al. 2020). S glycoprotein is a type I fusion protein which is related with virus tropism. This protein interacts with a human pulmonary cell surface receptor, Angiotensin-converting enzyme-2 (ACE2) which pays a role at SARS-CoV-2 entry (Zhou et al., 2020). Due to its function, interaction of S-protein and ACE2 molecule drawn attention. SARS-related coronaviruses (SARSr-CoV) binds to the ACE2 by receptor binding motif (RBM) located on their receptor binding domain (RBD) (Hulswit et al 2016, Letko et al 2020).

Origins for recently emerging coronaviruses

Alphacoronaviruses and betacoronaviruses infect mammals while gammacoronaviruses and deltacoronaviruses infect mammals as well as birds and fishes. Bats are linked as origin of alphacoronaviruses and betacoronaviruses, while usually swine are assumed as origin of gammacoronaviruses and deltacoronaviruses (Tiwari et al 2020). The SARS-CoV-2 is described as the seventh coronavirus infecting humans. The other coronaviruses which infect humans, some are defined as low pathogenic (HCoV-229E, HKU1, OC43, NL63) while SARS-CoV and MERS-CoV causing fatal pneumonia are highly pathogenic human coronaviruses (Liu et al 2020). Among those, SARS-CoV and MERS-CoV caused high mortality outbreaks since 2002, are in zoonotic character. Despite the main source for SARS-CoV have been mentioned as horseshoe bats (Rhinolophus sinicus) and civet cats (Paguma larvata), racon dogs (Nyctereutes procyonoides) and Chinese ferret badgers (Melogale moschata) were exerted as intermediate hosts (Lau et al 2005, Li et al 2006). MERS-CoV has also been originated from bats (Pipistrellus bat-CoV HKU5) (Woo et al 2012, Chan et al 2013) while dromedary (Arabian) camels were determined as the intermediate host (Liu et al 2020). In late 2019, “point zero” for starting COVID-19 cases was described as Huanan Sea Food Market where various exotic live animal species are sold. This information suggests that the new coronavirus may also jumped the species barrier and has an animal origin. By whole genome sequencing SARS-CoV-2 was found to be 96.2% identical to BatCoV RatG13 of bats (SARS-like bat coronavirus) (Paraskevis et al 2020, Li et al 2020). Average substitution rate for CoVs was estimated as 10^-4 /year /site. Beside the moderately high mutation rate in the largest RNA genome of coronaviruses provides the basis of genome modification by mutations and recombinations (Su et al 2016). Because of their RNA-dependent RNA polymerases (RdRP) lack of capability for correction (proof reading), genomic chances including mutations and recombinations frequently occur in coronavirus genome (Chen 2020). Thereby, virulence of circulating viruses, emergence of novel coronavirus species and “host jump” by interspecies transmission correlated with high mutation rates and genetic recombinations.

Possible intermediate hosts for SARS-CoV-2

Coronaviruses infect various domestic or wild animal species and human as well. Beside various clinical manifestations like enteritis (in cattle, horses and swine), upper respiratory tract disease (in dogs, feline, cattle, poultry) and fatal respiratory infections in human; usually infections can shown in subclinical status in most cases (Tiwari et al 2020). Among different animal species, coronaviruses have already been documented to cross the species barrier, as it was shown in SARS-CoV and MERS-CoV outbreaks. It is also assumed that SARS-CoV-2 initially originated from animal to human transmission and then human to human transmission followed (Hui et al 2020). Later, some questions have been raised for intermediate host range prospect.

Host tropism and distribution of an infectious virus is mainly related with the specificity of the virus and the cell surface
receptor interaction. Adaptation of coronaviruses to a new host is mainly associated with its S glycoprotein. Though the sequence and structural analyses of ACE2 are frequently preferred to recognize appropriate cell receptors to predict the susceptible host species. According to amino acid sequence alignments, it is proposed that SARS-CoV-2 may not interact with chicken ACE2, and non-human primates may be intermediate host for transmission (Li et al 2020). Pangolins, snakes and turtles have been also predicted as the potential intermediate hosts (Liu et al 2020). However involvement of snakes and turtles was contradicted and the idea of warm-blooded vertebrates are more likely to be an intermediate host than snakes had been suggested (Zhang et al 2020). But, indicating affinity of “pangolin ACE2” to SARS-CoV-2 supported that pangolins are involved in SARS-CoV-2 evolution (Luan et al 2020). By analyzing the protein sequences of mammals ACE revealed that the ACE2 molecules from Arabian camels, raccoon, greater horseshoe bat, brown rat, house mice (Mus musculus), platypus, African bush elephant, European hedgehog, raccoon dog, meerkats, Ord’s kangaroo rats and guinea pigs do not have binding capability to the RBD on S protein of SARS-CoV-2. But the same researchers suggested; some mammals including wild animals and pets (cats, dogs, horses, cattle, sheep, domestic pigs, rabbits, golden snub-nosed monkeys, rhesus macaques, weasels, masked palm civets, big-eared horseshoe bats, Chinese rufous horseshoe bats, Leschenault’s rousette, wild boars, ferrets, Pearson’s horseshoe bats, large flying foxes, Sumatran orangutan, chimpanzees, olive baboons, red foxes, Campbell’s dwarf hamsters, Syrian hamsters, Chinese hamsters, common marmosets, naked mole-rats, thirteen-lined ground squirrels) may be in the potential host list as well as pangolins (Luan et al 2020). Those of list includes some species (i.e. bats, monkeys, civets, dogs and cats) in which the infection has already been demonstrated, as well as another group of animals (i.e. sheep, cattle, horse, pigs, foxes, squirrels) in which there is no SARS-CoV-2 infection detected to date.

Household pets

As declared by World Organization for Animal Health (OIE), there are several animal species that have been tested positive for SARS-CoV-2, caused by close contact with people who are infected (OIE 2020). The animal species with confirmed natural SARS-CoV-2 infections are dog, cat, tiger, lion and mink.

Reports which demonstrates SARS-CoV-2 infection in dogs, laid out the infection caused by human-to-animal transmission. Two canine cases reported from different households in Hong Kong both were living in close contact with their COVID-19 infected owners (Sit et al 2020). Entirely, a 17 years old male Pomeranian dog was detected positive after owner was diagnosed for COVID-19. Although rectal swabs from the dog were negative, virus had been detected from nasal samp-les among 12 days. Secondly, 8 days after its owner’s confirmation for COVID-19, oral and nasal swab samples of the 2.5 years old German shepherd dog were detected PCR positive. In this case, positive result was detected only at the first day in fecal sample and, for 2 days in nasal and oral samples. Specific antibodies had been detected at both cases. But in a study analyzing experimental infections in different animal species, low susceptibility of dogs to SARS-CoV-2 was shown (Shi et al 2020). USDA’s National Veterinary Services Laboratories also confirmed cases of SARS-CoV-2 in dogs in the United States. PCR confirmed cases during June and August 2020 have been reported in the New York, Georgia, Texas, South Carolina, Arizona, Louisiana, and North Carolina (USDA APHIS 2020). Facing to a high titer virus shedding from infected people can be an important factor for the infection of the household dogs under natural conditions.

Serological screening in cat samples which were living in shelters or pet hospitals after COVID-19 outbreak in Wuhan, China demonstrated that cat population had also been infected in Wuhan during the SARS-CoV-2 outbreak (Zhang et al 2020). Till now, positive cases in cats which are living with SARS-CoV-2 infected owners have been reported in Hong Kong, New York- USA and Belgium (Shi et al 2020). But in April 22, 2020; two cats exhibiting mild respiratory illness in USA were confirmed positive for SARS-CoV-2, where susceptibility of the cats for airborne SARS-CoV-2 transmission is reported (Shi et al 2020). Later, transmission of the virus between inoculated and naive cats by direct contact was also demonstrated (Halfmann et al 2020). At the same experiment, nasal shedding from infected cats was documented however no virus detection was observed from rectal swabs. Despite efficient virus detection, none of the experimented cats had showed clinical symptoms. Studies reporting mild clinical signs including respiratory and digestive symptoms in infected cats is also available (Sailleau et al 2020).

Another important query existed so far is possibility of serological cross reaction between feline coronaviruses and SARS-CoV-2. Previously, to enlighten the interspecies transmission of coronaviruses within / between animal and human populations, 137 cat sera were analyzed for the presence of antibodies to CoV spike protein (Zhao et al 2019). According to the results 78 cats were positive for at least one or more coronavirus antibodies. Although cross-reaction was observed between feline coronavirus (FcCoV) and porcine epidemic diarrhea virus (PDEV) / PDVCoV, no seropositivity was observed against MERS-CoV and SARS-CoV. In a recent study, hyperimmunized sera produced against type I and II feline infectious peritonitis virus (FIPV) showed no cross-reactivity with SARS-CoV-2 (Zhang et al 2020). More detailed future studies are required to clarify exact situation for serological relationship between SARS-CoV-2 and coronaviruses of pet animals.
Possible role of abandoning domestic cats to street on the risk of infection in human is also studied on computer simulation (Gao et al 2020). According to this study, leaving domestic cats to the street can increase the risk of infection for humans. Though it is indicated that abandoning domestic cats for the fear of infection may cause increasing number of human infections.

Associations with other animal species

In a study which investigates serum samples of 35 animal species, including pigs, cattle, horses, sheep and poultry, detected all 1,914 sera as negative by SARS-CoV-2 specific antibody ELISA (Deng et al 2020). Besides, SARS-CoV-2 was detected in two mink farms in The Netherlands and in The United states as well (USDA APHIS 2020). Oreshkova and colleagues reported SARS-CoV-2 positive workers were also found in the mink farms in The Netherlands. Nasal discharge, severe respiratory distress and increased mortality were reported as clinical observations in the minks. Presence of viral RNA was demonstrated in all the collected throat samples of 36 necropsied animals and 34 rectal samples, also genomic sequences were presented. This report demonstrated susceptibility of mink and also transmission of the virus among each other. But the infection of farmers were assumed to have attracted from mink (Oreshkova et al 2020).

There is no case of SARS-CoV-2 in farm animals reported so far. SARS-CoV-2 detected in a Malayan tiger in New York (Wang et al 2020). While only cough indicated as clinical signs, in that case viral RNA detected in nasal, tracheal and oropharyngeal swabs. Though it seems that wildlife animals are also involved in disease existence rather than experimental studies. Updated animal information which involved in SARS-CoV-2 circulation or experiments are listed in Table 1.

Animal models for SARS-COV-2 research

Animal models are important tools for understanding pathophysiology of SARS-CoV-2 infections as well as development of efficient therapeutics and vaccines. According to amino acid sequence comparment it was shown that mice had the lowest homology. And also binding affinity of ACE2 from rabbits and mice has the lowest affinity comparing to cats, cattle, monkeys, dogs, pigs, horses, and sheep. Based on these data, it has been determined that natural mice and rats are not suitable for use in animal modeling studies under natural conditions (Shen et al 2020). But transgenic mice which are transhected with human ACE2 (hACE2) currently used in COVID-19 studies and exhibit clinical signs including weight loss and interstitial pneumonia (Takayama 2020). By that kind of studies, passively transferred neutralizing monoclonal antibody which obtained from hACE2-transduced mice reduced inflammation and viral burden in lung (Hassan et al 2020). In contrast to the computer modeling study mentioned above, a recent study (Mykytyn et al 2020) reported that rabbits are also susceptible to infection, and shedding was also detected even if clinical findings were not detected.

In the search of ACE2 interactions with SARS-CoV-2 the group of rodents in Circetidae (Mesocricetus auratus [golden hamster], Phodopus campbelli [dwarf hamster], Cricetulus

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Level of susceptibility</th>
<th>Type of infection</th>
<th>Exhibiting the symptoms</th>
<th>Transmission between animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dogs</td>
<td>Low</td>
<td>Natural and experimental</td>
<td>In some cases</td>
<td>Not reported</td>
</tr>
<tr>
<td>Cats</td>
<td>High</td>
<td>Natural and experimental</td>
<td>In some cases</td>
<td>Yes (between cats)</td>
</tr>
<tr>
<td>Pigs</td>
<td>None</td>
<td>Experimental</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Poultry</td>
<td>None</td>
<td>Experimental</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Tiger and lion</td>
<td>High</td>
<td>Natural</td>
<td>Yes</td>
<td>Yes (between animals)</td>
</tr>
<tr>
<td>Macaques</td>
<td>High</td>
<td>Experimental</td>
<td>Yes</td>
<td>Yes (between animals)</td>
</tr>
<tr>
<td>Ferret</td>
<td>High</td>
<td>Experimental</td>
<td>In some cases</td>
<td>Yes (between ferrets)</td>
</tr>
<tr>
<td>Mink</td>
<td>High</td>
<td>Natural</td>
<td>Yes</td>
<td>Yes (between minks)</td>
</tr>
<tr>
<td>Syrian hamster</td>
<td>High</td>
<td>Experimental</td>
<td>In some cases</td>
<td>Yes (between hamsters)</td>
</tr>
<tr>
<td>Egyptian fruit bats</td>
<td>High</td>
<td>Experimental</td>
<td>No</td>
<td>Yes (between fruit bats)</td>
</tr>
</tbody>
</table>

*The data adapted from OIE technical factsheet, 3 July 2020 (OIE 2020)
Table 2. Some experimental animal models for SARS-CoV-2 studies

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Level of susceptibility</th>
<th>Virus shedding (experimental infection)</th>
<th>Exhibiting clinical symptoms</th>
<th>Lung pathology</th>
<th>Model studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macaques</td>
<td>High</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>V,D,P,I</td>
</tr>
<tr>
<td>Ferrets</td>
<td>High</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>V,D,P,I</td>
</tr>
<tr>
<td>Syrian hamster</td>
<td>High</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>V,D,P,I</td>
</tr>
<tr>
<td>Cats</td>
<td>High</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>P,I</td>
</tr>
<tr>
<td>Trangenic (ACE2+) Mice</td>
<td>Low</td>
<td>na</td>
<td>na</td>
<td>Yes/mild</td>
<td>V,I</td>
</tr>
<tr>
<td>BALB/c mice*</td>
<td>Low</td>
<td>na</td>
<td>na</td>
<td>Yes/mild</td>
<td>V,I</td>
</tr>
<tr>
<td>Rabbits</td>
<td>Low</td>
<td>Yes</td>
<td>No</td>
<td>Yes/mild</td>
<td>V,I</td>
</tr>
<tr>
<td>Guine pigs</td>
<td>Low</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>V,I</td>
</tr>
</tbody>
</table>

*V: vaccine, D: Drug, P: Pathogenicity, I: Immunogenicity, na: No satisfied data available on date

*: by adaptation of virus

*griseus* [Chinese hamster] and *Ictidomys tridecemlineatus* [thirteen-lined ground squirrel] it was found to be capable of recognition of RBD (Luan et al 2020). Regarding the binding patterns between the receptors of various mammals and the SARS-CoV-2 S protein, Chinese hamsters and golden hamsters referred as possible small animal models (Luan et al 2020). Chimpanzees and monkeys shown to be most susceptible to SARS-CoV-2 than cats, mice, rats, and dogs, (Shen et al 2020).

Several different animal species are tried to be used in experimental studies. Animals that have been successfully used in modelling so far are Syrian hamsters, ferrets, cats, *Cynomolgus* macaques, and Rhesus macaques. Clinical signs of viral pneumonia had successfully examined in those animal species (Chandrashekar et al 2020, Kim et al 2020, Rockx et al 2020, Shi et al 2020). In an experimental study, productive upper respiratory tract infection in ferrets was evolved after intranasal inoculation and long term exposure (up to 11-19 days) has been documented (Richard et al 2020). At the same study direct or aerosol transmission were also conducted between donor ferrets and the naive ones (Richard et al 2020). Besides, Rhesus monkeys have also been used for efficiency testing of antiviral drug *Remdesivir* (Williamson et al 2020), adenovirus-vectored vaccine (Doremalen et al 2020) and DNA vaccine candidate (Yu et al 2020).

Pigs, chickens and ducks were also investigated for susceptibility to SARS-CoV-2. Intranasal inoculations were performed to ducks, chickens and pigs, then co-housed with non-inoculated animals. But no viral RNA or seropositivity were detected in any swabs of virus inoculated or naive contact animals (Shi et al 2020). Pigs and chickens were also referred as susceptible to intranasal infection by SARS-CoV-2 in another study. All the swab and organ samples of inoculated animals and also contact animals detected free for the virus (Promed 2020). These data suggesting that mentioned animals are not susceptible to SARS-CoV-2. However number of experiments and animal size are limited to create a certain conclusion.

Conclusion

COVID-19 has been declared as international public health emergency by WHO, due to the high transmissibility across the continents. Compared to SARS-CoV and MERS-CoV which also caused severe acute respiratory distress (SARD) outbreaks, COVID-19 has lower case fatality rate but has infected wide-range of the population. The outbreak not only effected human health or had economic impact but also created panic among animal owners and general public as well. Similarities between SARS-CoV and SARS-CoV-2 epidemiology and genetic identity resulted in euthanasia of dogs in China or abandoning the pets from homes by their owners in most countries. Before predicting an animal as an virus reservoir (pangolin, dogs, cat or turtles) it has to be guided by established principles like Koch’s postulates (Brownlie 2020). There are evidences of infections for some animal species which has close contact with SARS-CoV-2 infected people. For determining the control strategies in the context of one-health approach, it is important to know the main or intermediate hosts which have role for initial transmission or circulation. There are still small number of experimental studies with small sample size, which aims to show the relationship of intermediate hosts. It should be taken into consideration that the results obtained from experiments with small number size can only give an idea about the circulation and susceptible conclusions may change in the future. Though further studies are required to enlighten the roles of recommended species in SARS-CoV-2 circulation or their contribution to studies.
Conflict of Interest

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During this study, any pharmaceutical company which has a direct connection with the research subject, a company that provides and/or manufactures medical instruments, equipment and materials or any commercial company may have a negative impact on the decision to be made during the evaluation process of the study or no moral support.

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References

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