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## Impact of covid-19 on molecular cytogenetics in domestic animal studies: A mini-review

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### Abstract

Coronavirus diseases originated from Wuhan, China has emerged as a global pandemic and impacted all sort of human activities including scientific research. Coronaviruses are zoonotic in nature, but there is lack of evidence to explain COVID-19's original route of transmission from animal to human sources. Many animals like big cats, non-human primates, dogs, ferrets, and minks have been tested positive for COVID-19. Veterinary Services have greatly contributed to support the international and national response to COVID-19. It has pushed the global economy and had devastating on the living conditions on a global scale. COVID-19 pandemic has devastated many industries like aviation, tourism, event management, etc., but at the same time importance of research funding had been realized globally. Cytogenetics has also observed new research projects particularly aimed at SARS-CoV-2 diagnosis. Cytogenetics study proved to be a very accurate tool for tissue specific diagnosis of the virus. Detection of viral antigen using IHC or immunofluorescence assay (IFA) techniques and detection of viral nucleic acids using in situ hybridization (ISH) within infected, but inactivated, human or animal model tissues greatly facilitate detection of viral infection. Research in animal cytogenetics is being promoted in this COVID-19 era, maybe because of global realization of importance of research funding due to sudden pandemic situation, its utilization in accurate diagnosis and awareness among scientific community for One health collaborations.

**Keywords:** covid-19, molecular cytogenetics, domestic animal studies

### Introduction

The infection brought about by the novel corona virus which was initially recognized in Wuhan, China, has been named as coronavirus disease 2019 (COVID-19). The infection is communicated through direct contact with respiratory drops of infected individuals, and contacting surfaces debased with the infection (UNICEF).

There is a long-documented history of zoonosis that turned into a pandemic. The U.S. Center for Disease Control and Prevention stated that more than 6 out of every 10 known infectious diseases in people can be spread from animals, and 3 out of every 4 new or emerging infectious diseases in people come from animals (CDC). The majority of severe pandemics are caused by close contact between humans and animals, and the proportion of zoonosis transmitted by farm animals rather than wild animals has steadily increased over the years (Jones *et al.* 2008) [7]. The intensifying emergence of infectious pathogens is driven by the growing anthropogenic impact on nature and can be attributed, among other factors, to biodiversity loss and habitat degradation.

COVID-19 is a novel disease caused by a newly identified virus, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). The outbreak was initially noticed in a seafood market in Wuhan city in Hubei Province of China in mid-December, 2019, which spread to 215 countries/territories/areas worldwide. It was declared as "Public Health Emergency of International Concern" (PHEIC) by WHO on 30th January 2020 and subsequently declared as a pandemic on 11th March, 2020. Coronaviruses are zoonotic meaning that the viruses are transmitted between animals and humans. It has been reported that MERS-CoV was transmitted from dromedary camels to humans and SARS-CoV from civet cats to humans (Chan *et al.*, 2015) [3]. The origins of COVID-19 virus are also believed to be from animal source. The genomic sequence data reveals 80% similarity between the SARS-CoV-2 and the previous human coronavirus (SARS-CoV), establishing its close relation to the *Rhinolophus bat* (Horseshoe bat) population. There is also a possibility of the involvement of an intermediate host for transmission to humans.

Health officials have found the first SARS-CoV-2 infections to be linked to the live animal market in China (Liu and Saif, 2020) [10]. Based on various reports, it is concluded that SARS-CoV-2 has a zoonotic source closely related to bat-origin SARS-like coronavirus having enveloped RNA beta coronavirus, shown to use the angiotensin-converting enzyme 2 (ACE2) receptor for cell entry (www.mohfw.gov.in). Affirming the similarity, it is likely that bats serve as reservoir hosts for its progenitor. Although RaTG13, sampled from a *Rhinolophus affinis* bat1, is ~96% identical overall to SARS-CoV-2, its spike diverges in the RBD, suggesting that it may not bind efficiently to human ACE27. The researchers of Agricultural university of South China analyzed over one thousand metagenomic samples and found 70% pangolins were positive for the coronavirus (Lu *et al.*, 2020) [11]. Similarity of the genetic sequence between the pangolins and the present infectious human strain was 99% (Zhang and Holmes, 2020) [20]. These pangolins were imported illegally from the Guangdong and Guangxi provinces of Southern China (Lake, 2020) [8]. In another study (Yadav *et al.*, 2020) [19], bat samples from seven different states of India, the rectal swab test of eight samples of *Rousettus* spp. and *Pteropus* spp. were positive for RdRp gene where, the results of the next generation sequencing revealed that the four sequence regions of *Rousettus* spp. had about approximately 94.3% similarity to the previously found coronaviruses in bat (Yadav *et al.*, 2020) [19]. White camels are thought to be the true reservoir hosts from MERS (de Wit *et al.*, 2016) [4]. However, till date, there are not enough scientific documental evidence to explain the original route of transmission from animal to human sources. It is a possibility that the progenitor of SARS-CoV-2 jumped into humans, acquiring the genomic features through adaptation during undetected human-to-human transmission. This enabled the pandemic to take off and produce a sufficiently large cluster of cases to trigger the surveillance system that detected it (Zhou *et al.*, 2020; Wu *et al.*, 2020) [21, 18]. The transmission of viruses of beta ( $\beta$ ) genera from animals to humans had harmful consequences in the past (Guarner, 2020) [5]. In 2002–2003, in China into Guangdong province, a beta coronavirus originated in bats moved to human via palm civet cats which acted as their intermediate host (Martina *et al.*, 2003) [12]. A decade later, in 2012, another coronavirus named, Middle East Respiratory Syndrome coronavirus, MERS-CoV originated in Saudi Arabia, transmitted from bats with dromedary camels being the intermediate host (Qian *et al.*, 2013) [15]. These infected about 2494 people with 858 fatalities. The first US case of an animal testing positive for COVID-19 was a tiger at a New York zoo. Several other wild animals in zoos and sanctuaries have tested positive for SARS-CoV-2, including big cats (lions, tigers, pumas, cougars, snow leopards) and non-human primates (gorillas) after showing signs of illness. SARS-CoV-2 has also been reported in mink on various farms (www.cdc.gov). Infection with SARS-CoV-2 has been reported in mink in numerous countries worldwide, including the Netherlands, Italy, Spain, France, Canada and the United States. Reports from affected mink farms show an increase in respiratory diseases and deaths, although in some cases, asymptomatic mink from affected farms have also tested positive. Among domestic animals, SARS-CoV-2 has been confirmed in cats, dogs, and ferrets. Confirmed cases in pets are extremely rare, and fortunately result in mild or no clinical signs of illness. There have been no documented cases of COVID-19 infection in horses or

livestock species, and there is no evidence to date that humans represent a risk of this infection to farm animals (vet.osu.edu). The COVID-19 pandemic has provided new evidence that a longstanding and sustainable one Health collaboration is needed. Beyond collaborative research activities, the animal health sector has contributed in various ways towards building a common response to the pandemic in the field. The veterinary profession has shown its commitment to support the work of human health authorities. Whether for the provision of testing capacity by animal health laboratories, through donating essential materials such as personal protective equipment and ventilators, or through direct provision of human resources and expertise, Veterinary Services have greatly contributed to support the international and national response to COVID-19 (World Organisation for Animal Health).

The “black swan” metaphor is used for a highly improbable and, thus, virtually unpredictable event, which, when it materializes, has a disruptive impact on the markets. The COVID-19 pandemic can be considered as such a “black swan” event, which not only pushed the global economy in dire straits but primarily had devastating effects on the living conditions on a global scale (Simianer and Reimer, 2021) [16]. COVID-19 pandemic has devastated many industries like aviation, tourism, event management, etc., but at the same time importance of research funding had been realized globally. Massive research projects have been initiated related to solve the problems related to COVID-19, like early diagnosis, effective treatment and accurate prognosis. The research work in these field picked up the speed in early 2020 itself when COVID-19 was in emerging stage. The area of “cytogenetics” has also observed new research projects particularly aimed at SARS-CoV-2 diagnosis. Cytogenetics study proved to be a very accurate tool for tissue specific diagnosis of the virus. COVID-19 regulations made daily business more complicated and possibly less profitable but, did not threaten R&D industry overall, although it uplifted certain research industries directly related to COVID-19 pandemic.

In humans, pre-natal and post-natal genetic counselling holds an important place in developed countries. Cytogenetics is an important tool in this process which helps in diagnosis of rare genetic disorders. Spread of SARS-CoV-2 virus infection had forced to change the working habits. Improvement of real-time video conferencing with high-speed technologies, tele-genetics and tele-medicine consultation proved to be an effective method to provide genetic counseling.

SARS-CoV-2 has a non-segmented, linear, positive-sense, multi-cistronic genome and produces enveloped virions. The virus is classified as a betacoronavirus (*Nidovirales: Coronaviridae*). Small animals, for example, human ACE2–transgenic laboratory mice, cats, domestic ferrets, golden hamsters, and nonhuman primates (e.g., rhesus monkeys, crab-eating macaques) are used to study SARS-CoV-2 infection, as alveolar damage, interstitial inflammation, and viral shedding occur in these animal models to various degrees (Muñoz-Fontela *et al.*, 2021) [13]. Such studies involving animal cytogenetics have been escalated in the COVID-19 pandemic.

Detection of viral antigen using IHC or immunofluorescence assay (IFA) techniques and detection of viral nucleic acids using in situ hybridization (ISH) within infected, but inactivated, human or animal model tissues greatly facilitate detection of viral infection and thereby pathogenesis and

MCM (medical counter measures) efficacy studies (Liu *et al.*, 2020, Rocha *et al.*, 2020) [10, 11]. These techniques become paramount in particular for studies of a potential pathogen that does not cause overt, or causes only mild, disease, such as SARS-CoV-2 in the currently available animal models.

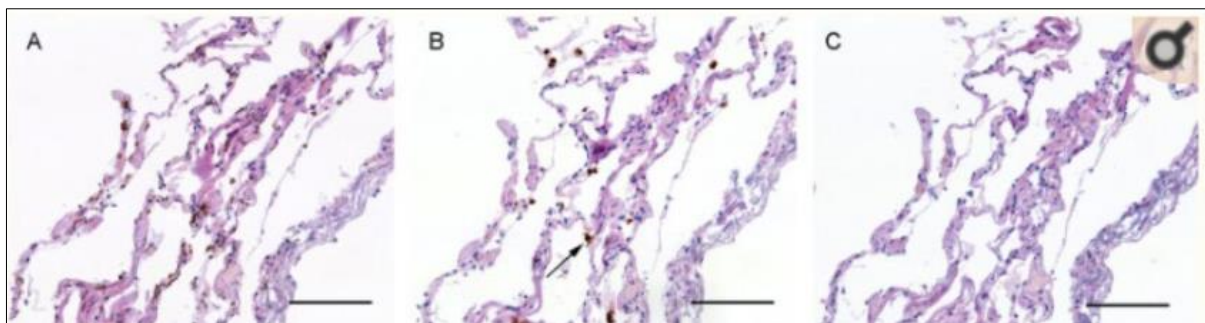
In situ hybridization (ISH) assays can be used for tissue identification of the virus. Rocha and co-workers (2020) [11] performed RNA ISH on the Leica BOND-III platform (Leica, Wetzlar, Germany) using RNAScope probes (ACD, Newark, CA) directed against SARS-CoV-2, targeting 21631–23303 base pairs (Fig.1).

In another study (Liu *et al.*, 2020) [9] ISH and multiplex-FISH (mFISH) (Fig. 2) was established to detect SARS-CoV-2 RNA. ISH, will be essential in understanding organ involvement and cell-type specific infections by SARS-CoV-2. ISH assays represent a sensitive and specific method for detecting the virus in tissue samples.

Dimensions platform ([www.dimensions.com](http://www.dimensions.com)) can be used to search the keywords in all the related publications, ranging

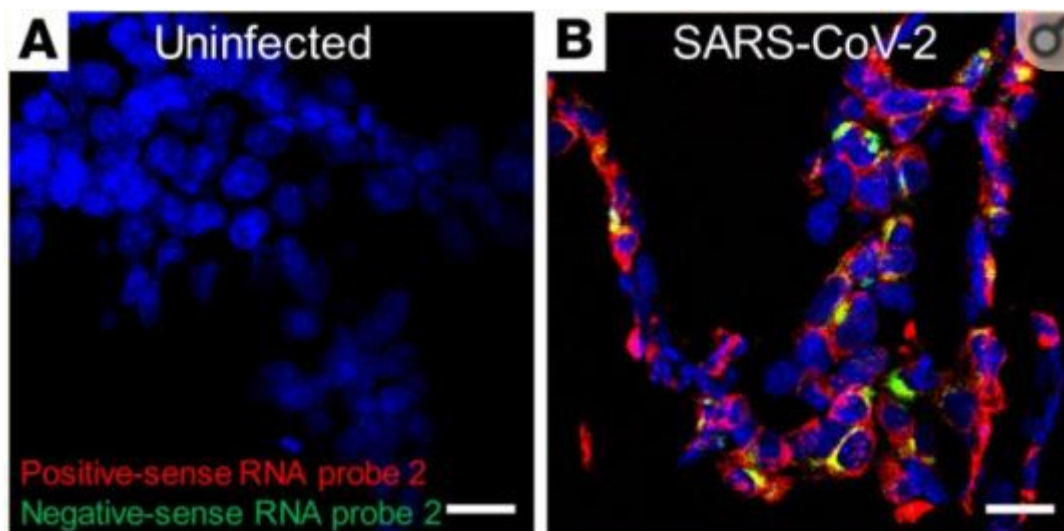
from articles published in scholarly journals, books and book chapters, to preprints and conference proceedings. When the keyword “cytogenetics” was hit for the search in “Titles and Abstracts” of the publications, year-wise data was obtained for the related publications. The first case of COVID-19 was seen in November 2019 but its effect was observed from 2020. Little growth was seen from 2018 to 2019 but publications were almost equal for year 2019 and 2020. More than half a year of 2021 has passed with 1842 publications (Fig. 3), and with same speed we can expect around 2000-2500 publications for the present year.

The search results were filtered for cytogenetics work in veterinary sciences, and a growth was observed from 2019 to 2020, indicating increased livestock cytogenetics studies. For 2021, till now there are 28 publications (Fig. 4) and with same growth we can expect 35+ publications which will be a continuation in the upward trend. Fig.5 indicates veterinary and allied subject's cytogenetics associated publications in the COVID era (2020 and 2021).



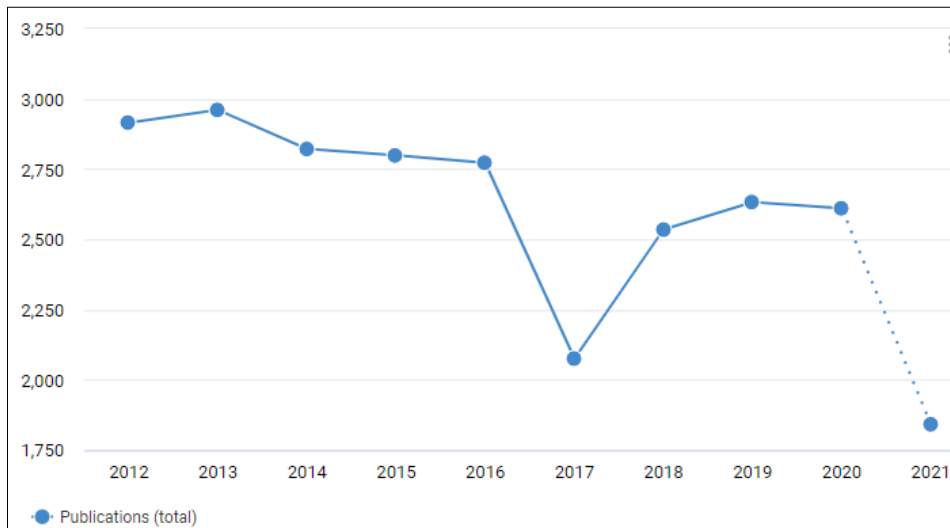
Positive reaction for the (a) control probes to the housekeeping gene peptidylprolyl isomerase B (PPIB) by in situ hybridization confirms the presence of intact nucleic acid. (b) There is a positive reaction for the probes directed against SARS-CoV-2 (arrow) and (c) a negative reaction for the negative control probes (bacterial gene *dapB*) in a serial section.

**Fig 1:** ISH in alveolar tissue (Reprinted from “Detection of SARS-CoV-2 in formalin-fixed paraffin-embedded tissue sections using commercially available reagents” by Rocha *et al.*, 2020)

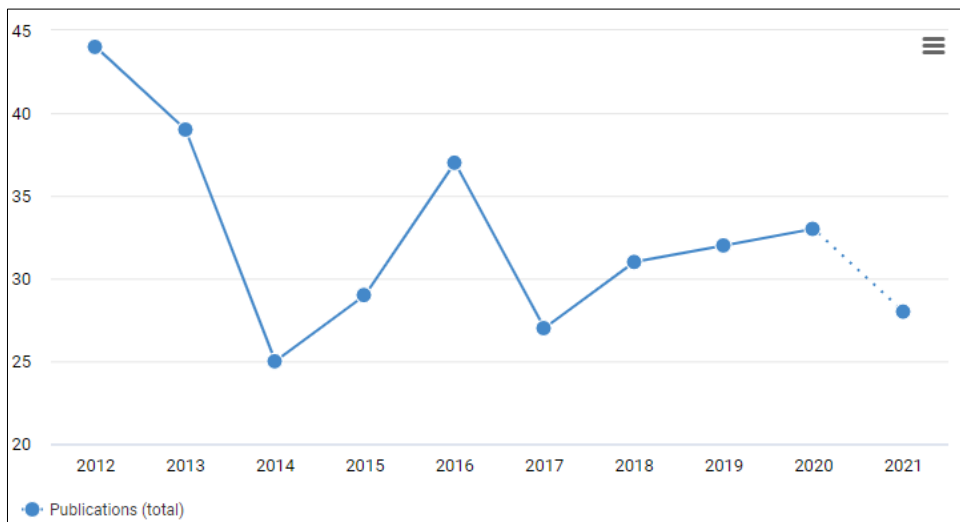


(A and B) Compared with uninfected control (A), SARS-CoV-2 negative-sense RNA (green), a replicative intermediate that indicates viral replication, can be detected in infected cell pellets in addition to positive-sense (red) RNA (B). Nuclei are stained blue (DAPI).

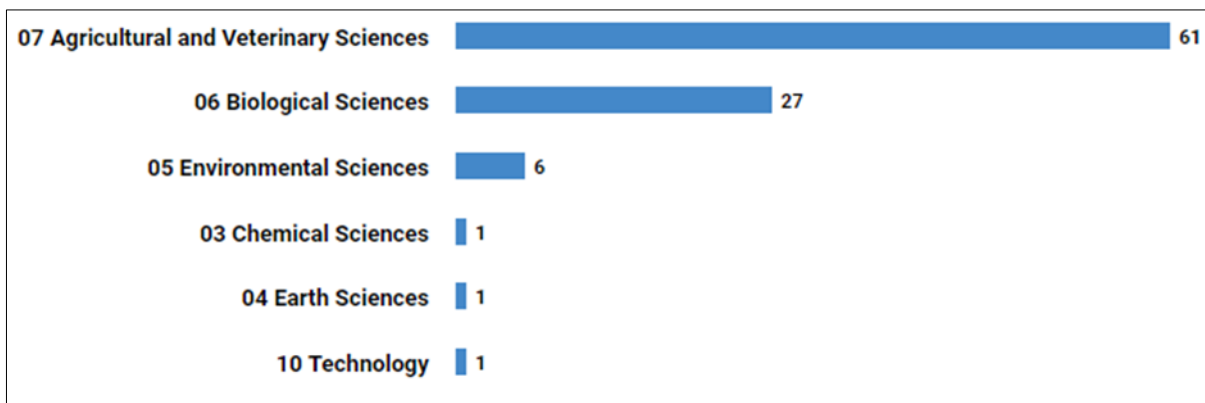
**Fig 2:** Detection of SARS-CoV-2 replication using multiplex FISH (Reprinted from “Molecular detection of SARS-CoV-2 in formalin-fixed, paraffin-embedded specimens” by Liu *et al.*, 2020)



**Fig 3:** Number of overall cytogenetic related studies (Adapted from <https://www.dimensions.ai/>)



**Fig 4:** Number of cytogenetic studies in veterinary science ((Adapted from <https://www.dimensions.ai/>)



**Fig 5:** Veterinary and related subjects with cytogenetics associated publications in 2020 and 2021 (Adapted from <https://www.dimensions.ai/>)

**Conclusion**

Above data indicates the research in animal cytogenetics is being promoted in this COVID era, maybe because of global realization of importance of research funding due to this sudden pandemic situation, its utilization in detection of viral infection, pathogenesis and medical counter measures efficacy studies, along with the awareness among scientific community that a longstanding and sustainable One Health collaboration is required to tackle such situation.

**References**

1. Best Rocha A, Stroberg E, Barton LM, Duval EJ, Mukhopadhyay S, Yarid N, *et al.* Detection of SARS-CoV-2 in formalin-fixed paraffin-embedded tissue sections using commercially available reagents. *Laboratory Investigation* 2020;100(11):1485-1489. <https://doi.org/10.1038/s41374-020-0464-x>
2. Centers for Disease Control and Prevention. <https://www.cdc.gov/onehealth/basics/zoonotic->

- diseases.html (accessed on October 22, 2021)
3. Chan JF, Lau SK, To KK, Cheng VC, Woo PC, Yuen KY. Middle East respiratory syndrome coronavirus: another zoonotic betacoronavirus causing SARS-like disease. *Clinical microbiology reviews* 2015;28(2):465-522.
  4. de Wit E, van Doremalen N, Falzarano D, Munster VJ. SARS and MERS: recent insights into emerging coronaviruses. *Nat Rev Microbiol* 2016;14:523-534. doi: 10.1038/nrmicro.2016.81
  5. Guarner J. Three emerging coronaviruses in two decades: the story of SARS, MERS, and now COVID-19. *Am J Clin Pathology* 2020;153:420-421. doi: 10.1093/ajcp/aqaa029.
  6. <https://doi.org/10.1038/s41586-020-2012-7>
  7. Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, *et al.* Global trends in emerging infectious diseases. *Nature* 2008;451(7181):990-993. <https://doi.org/10.1038/nature06536>
  8. Lake MA. What we know so far: COVID-19 current clinical knowledge and research. *Clinical Medicine* 2020;20:124-127. doi: 10.7861/clinmed.2019-coron.
  9. Liu J, Babka AM, Kearney BJ, Radoshitzky SR, Kuhn JH, Zeng X. Molecular detection of SARS-CoV-2 in formalin-fixed, paraffin-embedded specimens. *JCI Insight* 2020;5(12):e139042. <https://doi.org/10.1172/jci.insight.139042>
  10. Liu SL, Saif L. Emerging viruses without borders: the Wuhan coronavirus. *Viruses* 2020;12:130. doi: 10.3390/v12020130.
  11. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, *et al.* Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet* 2020;395:565-574. doi: 10.1016/S0140-6736(20)30251-8.
  12. Martina BEE, Haagmans BL, Kuiken T, Fouchier RAM, Rimmelzwaan GF, van Amerongen G, *et al.* SARS virus infection of cats and ferrets. *Nature* 2003;425:915-9.
  13. Muñoz-Fontela C, Dowling WE, Funnell SGP *et al.*, Animal models for COVID-19 *Nature* 2020;586:509-515. <https://doi.org/10.1038/s41586-020-278>
  14. Public Health England. COVID-19: epidemiology, virology and clinical features. Available from: <https://www.gov.uk/government/publications/wuhan-novel-coronavirus-background-information/wuhan-novel-coronavirus-epidemiology-virology-and-clinical-features>. (Accessed October 20 2021)
  15. Qian Z, Dominguez SR, Holmes KV. Role of the spike glycoprotein of human Middle East respiratory syndrome coronavirus (MERS-CoV) in virus entry and syncytia formation. *PLoS One* 2013;8:5-10.
  16. Simianer H, Reimer C. COVID-19: A “black swan” and what animal breeding can learn from it. *Animal Frontiers* 2021;11(1):57-59. <https://doi.org/10.1093/af/vfaa046>
  17. World Health Organization. Coronavirus. Available from: <https://www.who.int/health-topics/coronavirus> (Accessed October 20 2021)
  18. Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, *et al.* A new coronavirus associated with human respiratory disease in China. *Nature* 2020;579(7798):265-269. <https://doi.org/10.1038/s41586-020-2008-3>
  19. Yadav PD, Shete-Aich A, Nyayanit DA, Pardeshi P, Majumdar T, Balasubramanian R, *et al.* Detection of coronaviruses in Pteropus & Rousettus species of bats from different states of India. *Indian J Med Res* 2020;151:226-235.
  20. Zhang YZ, Holmes EC. A genomic perspective on the origin and emergence of SARS-CoV-2. *Cell* 2020;181:223-227. doi: 10.1016/j.cell.2020.03.035.
  21. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, *et al.* A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020;579(7798):270-273.
  22. COVID-19 and Animals, The Ohio State University (<https://vet.osu.edu/about-us/news/covid-19-and-animals>, accessed on 21/10/2021)
  23. Animals and COVID-19, Centers for Disease Control and Prevention (CDC) (<https://www.cdc.gov/coronavirus/2019-ncov/daily-life-coping/animals.html>, accessed on 21/10/2021)
  24. DIMENSIONS (<https://www.dimensions.ai/>, accessed on 31/10/2021)
  25. COVID-19, World Organization for Animal Health (OIE) (<https://www.oie.int/en/what-we-offer/emergency-and-resilience/covid-19>)
  26. Clinical management protocol for COVID-19 (for adults), Ministry of Health and Family Welfare, Govt. of India ([www.hohfw.gov.in](http://www.hohfw.gov.in), accessed on 21/10/2021)
  27. ([www.mohfw.gov.in](http://www.mohfw.gov.in), accessed on 21/10/2021)
  28. All you need to know about Coronavirus in India, United Nations Children's Fund (UNICEF) (<https://www.unicef.org/india/coronavirus/covid-19>, accessed on 21/10/2021)