Delaney Yorks
October 17, 2020
Junior Seminar
Final Paper

Overview of COVID-19 and Developing Information Regarding Animal Models, Mental Health and Vaccine Advancements

Abstract

The novel coronavirus (SARS-CoV-2) has negatively impacted our nation and world. Due to the rapid spread of COVID-19 our nation was forced into a mandatory quarantine. The mandatory quarantine changed many of our livelihoods, and we are still experiencing the same problems. For the first time in our lifetimes, we were forced by fear and worry to stay in our homes for weeks and only come in contact with immediate family and friends. During this time the SARS-CoV-2 virus spread across the world killing thousands of people and infecting millions. COVID-19 was declared a global pandemic on March 11, 2020 by the World Health Organization, and the race for a safe and effective vaccine was on. New discoveries about SARS-CoV-2 have been made as a result of the hard work of health care workers and scientists. The examination of similar coronaviruses, vaccine technology and animal models all contributed to developing new information regarding this deadly virus.

Coronavirus

The SARS-CoV-2 virus has taken 258,000 lives in the United States and as of November 24, 2020 2.5 million have been recorded. The pandemic that we are experiencing is due to a pneumonia-like respiratory virus known as SARS-CoV-2. The "SARS" acronym in the name of this coronavirus stands for Severe Acute Respiratory Syndrome, indicating that the virus replicates in the upper respiratory system. There have been outbreaks of similar coronaviruses in other parts of the world. From 2002-2003 there was an outbreak of the SARS-CoV in China, and there was an outbreak of the MERS-CoV in the Arabian Peninsula in 2012 (Mohammadi et al. 2020). These outbreaks were similar to that of the current SARS-CoV-2 outbreak. The SARS-CoV, MERS-CoV and SARS-CoV-2 are all classified as coronaviruses, due to the appendage-like extensions coming off of the virus molecule giving it a crown-like appearance, hence the name "coronal" meaning crown (Mohammadi et al. 2020). However, the present coronavirus outbreak is on a larger scale and has impacted many more lives worldwide.

The second part of the scientific name of the SARS-CoV-2 (CoV) stands for coronavirus. Coronaviruses have a high mutation rate and also contain zoonotic pathogens which can be transmitted between animals and humans through close contact, being bitten by an infected animal, eating unsafe foods or drinking contaminated water (CDC 2020). These pathogens can cause clinical problems in the respiratory, gastrointestinal, hepatic, and neurologic systems. Zoonotic pathogens also have a variety of asymptomatic strains that can lead to the infection of others (Mohammadi et al. 2020). All of these factors mentioned above are major contributors which explain why this coronavirus is so dangerous and can spread so rapidly. Figure 1 shows the analysis of the number of cases that were reported as of March 1, 2020. This graph is a great visual representation of how quickly the virus spread in multiple continents across the world. Some contributing factors that have increased the spread are asymptomatic individuals spreading the virus without knowing that they are carriers, the virus can continue to live on surfaces after an infected individual touches something and respiratory droplet transmission can occur. As time goes on, more information has surfaced and has helped us control and diagnose individuals who have COVID-19.

COVID-19

SARS-CoV-2, otherwise known as COVID-19, presumably began at a local seafood market in Wuhan, China. This novel coronavirus was discovered by the Chinese Centers for Disease Control and Prevention (CDC) in a throat swab sample of a patient. On January 7, 2020 COVID-19 was declared as a new type of coronavirus, and the first viral genome sequence was released on January 10, 2020 (Mohammadi et al. 2020). The exact source of this novel coronavirus is still unknown, but it is believed that the virus was probably transmitted from an animal to a human (zoonotic agent). The second transmission was from human to human, which can be observed as the increase in the number of cases reported in China after the initial discovery of this coronavirus (Mohammadi et al. 2020). Symptoms of COVID-19 can include fever, cough, shortness of breath, fatigue, muscle or body aches, headache, loss of taste or smell, sore throat, congestion or runny nose, nausea, and or diarrhea (CDC 2020). The severity of symptoms and infection varies between individuals. Some individuals may have mild symptoms, while others might be in the intensive care unit of the hospital for days or months. It has also been noted that older individuals appear to be more severely impacted by COVID-19 than younger individuals. There are few or no treatment options in the sudden occurrence of a new viral disease. Currently, a vaccine is being researched and will hopefully be released soon. Some antiviral drugs have been used to evaluate their efficacy in treating COVID-19. For example, an antiviral drug called Remdesivir was used in the first case that was reported in the United States. Remdesivir was deemed successful because in-vitro studies have shown that viral RNA transcription was terminated in the early stages of replication, although more cases would need to be evaluated to further investigate the effectiveness of this drug (Mohammadi et al. 2020).

Since COVID-19 can spread rapidly as an airborne virus, the CDC suggests to stay six feet apart from other people to avoid close contact; wash your hands with hot water and soap or use a hand sanitizer that contains at least 60% alcohol; avoid touching your eyes, nose, and mouth; wear a mask when with others that are not your immediate family; cover coughs and sneezes; clean and disinfect frequently touched surfaces; and monitor health and symptoms daily (CDC 2020). Since COVID-19 is spread mainly from person-to-person it is vital to practice social distancing and wear a mask.

Animal Models

Animal models have been used in the past to research the replication and transmissibility of viruses. Animal models play an important role in determining the effectiveness of potential therapeutic strategies for COVID-19 (Lakdawala and Menachery 2020). Animal models are used to "understand disease progression, pathogenesis, and immunologic responses to viral infections in humans" (Ruiz et al. 2017). Animal models are also used to test vaccines, and they are essential for preclinical studies (Ruiz et al. 2017). One study in the pandemic investigated the susceptibility, replication, and transmissibility of COVID-19 performed on cats, ferrets, dogs, chickens, pigs and ducks (Shi et al. 2020). The animals that provided the most conclusive information from these studies were ferrets and cats. Mice are typically used for epidemiological studies due to low cost and high reproduction rates, but they could not be used as a possible animal model for COVID-19 because of their receptor incompatibility (Shi et al. 2020).

The purpose of studying replication and transmissibility of the virus in the selected animal models was to provide data about which individuals COVID-19 infects the most, how this virus replicates and to assess the immunologic responses of the animal models. Information regarding the replication and transmissibility of the SARS-CoV-2 virus in animals could help us understand how it will affect the human population. For this experiment, animal models were obtained and inoculated with either the CTan-H strain or the F13-E strain of SARS-CoV-2. The CTan-H strain was a sample collected from a human patient with SARS-CoV-2, and the F13-E strain was an environmental strain that was collected from Wuhan, China (Shi et al. 2020). The purpose of having two different strains was to see how the animal models reacted to each strain. Each of the strains was inoculated into the animal models intranasally at 10^5 plaque forming units (PFU). Animal models were analyzed after euthanasia by detecting viral RNA and viral titers. The detection of the viral RNA and viral titers helped determine where and how much each of the strains replicated in the animal models. (Shi et al. 2020).

The inoculation of the F13-E strain or CTan-H strain of SARS-CoV-2 in ferrets provided conclusive evidence regarding replication and transmissibility of COVID-19. The results of the study of replication of the two different strains conclusively showed that both the F13-E strain and the CTan-H strain replicated considerably in the upper respiratory system of the ferrets (Figure 2). More specifically, both strains replicated in the nasal turbinate, soft palate, and tonsils of the ferrets (Shi et al. 2020). Each strain replicated significantly compared to the original inoculated concentration of 10^5 PFU of each of the strains. This is similar to what has been observed in humans: SARS-CoV-2 replicates in the upper respiratory system.

Cats also showed conclusive evidence about the replication and transmissibility of the CTan-H strain. The replication of the CTan-H strain was examined in two different age groups of cats: juvenile and subadult cats. These two different age groups were chosen to be studied to examine if the effects of the virus differ between age groups. Figure 3 specifically analyzes subadult and juvenile cats that were euthanized six days after inoculation (Shi et al. 2020). The cats were euthanized on days 3, 6, and 12 to assess how quickly the virus replicates. It is important to note that the cats were euthanized on different days, but day 6 was selected for effective visualization of the proper analysis. The difference in the areas of replication between subadult and juvenile cats can be observed in Figure 3 (Shi et al. 2020); the CTan-H strain was detected in more areas in the juvenile cats than in the subadult cats. For example, the CTan-H strain was detected in the lungs at a greater concentration and a greater frequency in the juvenile cats than the subadult cats. The replication also occurred in the upper respiratory system for both of the age ranges. The fact that the CTan-H strain replicated more in the juvenile (younger) cats is contradictory to what has been observed in humans (Shi et al. 2020). In humans, it has been observed that SARS-CoV-2 replicates more in older individuals. This discovery could help us determine the extent of the replication of SARS-CoV-2 in humans.

A third component of the study by Shi et al. 2020 relates to the transmissibility of the CTan-H strain of the virus in subadult cats. In Figure 4 the analysis of respiratory droplet transmission is examined through experimenting with subadult cats (Shi et al. 2020). Three pairs of cats were analyzed in this part of the experiment. In each pair, one cat was inoculated with 10^5 PFU of the CTan-H strain and the other cat was exposed only to its inoculated pair. The pairs of cats were placed in adjacent enclosures ensuring that they could not physically touch but could possibly exchange virus particles through the air. Of the three pairs of cats, one pair experienced successful respiratory droplet transmission, which means that the inoculated cat transmitted the virus to the exposed cat through air transmission. The researchers came to this conclusion because after euthanasia of the cats in the study, viral RNA was detected in the cat that was exposed to its inoculated pair (Shi et al. 2020).

These findings in relation to the most appropriate animal model for SARS-CoV-2 can possibly help researchers understand how this virus replicates and how it is transmissible between humans and animals. It is worth noting that this study conducted by Shi et al. 2020 was published on April 8, 2020. This research was one of the first studies conducted on animal models regarding SARS-CoV-2.

Mental Health

The SARS-CoV-2 outbreak in the United States and around the world has negatively impacted millions of people's lives both physically and mentally. The present pandemic is leading to additional health problems such as stress, anxiety, depressive symptoms, insomnia, anger, and fear (Torales et al. 2020). Since the present concern is the physical health of our nation and world, mental health has been pushed to the side. The impact of the pandemic on global mental health has not been assessed in depth. Our current situation can be compared to the MERS-CoV outbreak in Korea in 2015 and also to the SARS-CoV outbreak in 2003 in Singapore. These previous coronavirus experiences can provide information about what individuals are experiencing during the present pandemic and what we need to prepare for (Torales et al. 2020). For example, it was observed that during the MERS-CoV outbreak patients experienced high levels of stress. This is supported by the delay in the levels of circulating cell-free genomic DNA and the circulating cell-free mitochondria DNA. The delay in these levels indicates psychophysical stress in humans (Torales et al. 2020). Also, staff involved with the MERS-related tasks showed post-traumatic stress disorder symptoms after the outbreak. An example from information gathered from the 2003 SARS-CoV outbreak in Singapore showed that 27% of health care workers reported psychiatric symptoms after enduring the stressful community environment (Torales et al. 2020). It has been reported that 41-65% of SARS survivors experienced persistent psychological symptoms. Also, it was found that healthcare workers who cared for SARS patients continued to experience substantial psychological stress 1-

2 years after the SARS-CoV outbreak (Maunder 2009). Not only were the health care workers experiencing stress, but all individuals who were involved were under extreme stress due to the fear of spreading the virus. In regard to the general population after the outbreak of SARS-CoV depression increased by 7% (Torales et al. 2020). This shows that the SARS-CoV and the MERS-CoV outbreaks effected the entire population not just healthcare workers.

Similar to the MERS-CoV and the SARS-CoV outbreaks, health care workers during the COVID-19 outbreak have endured great amounts of stress. This stress can be contributed to being exposed to the virus, not having effective protection, overwork, isolation, and a lack of contact with their families. These problems are causing a great decline in mental health. These mental health problems contribute to the lack of attention, understanding, and decision-making capacity of medical workers (Torales et al. 2020). These preliminary side effects of the current situation (stress, anxiety) can contribute to the effectiveness of health care workers, and therefore affect the overall outcome of our fight against COVID-19.

Vaccine

Vaccines are used to provide active immunity to a particular disease or virus. Usually, a vaccine contains an agent that resembles the disease-causing organism with dead virus particles in it. The vaccine stimulates the body's immune system to recognize disease-causing agents in the future so that the body will be able to fight off the illness once infected (CDC 2019). Vaccines can be hard to make and must be deemed safe and effective before they can be distributed to the public. Once a successful vaccine has been developed it can be distributed to the public as an effective method of preventing infectious diseases.

Previous experience with the MERS-CoV and SARS-CoV outbreaks provides us with some supporting information for the basis of the new vaccine that must be created. Two primary design strategies being used for the development of the COVID-19 vaccine that are being considered is the usage of the whole virus or genetically engineered vaccine antigens (Ong et al. 2020). The development of vaccines has been revolutionized by reverse vaccinology. This technique identifies possible vaccine candidates through bioinformatics analysis of the pathogen genome. In a research paper published in July of 2020, the Vaxign and the newly developed Vaxign-ML reverse technology tools were used to predict possible COVID-19 vaccine candidates (Ong et al. 2020). The investigation of the entire proteome of SARS-CoV-2 resulted in finding six proteins that are predicted to be adhesins. Adhesins are crucial to the viral particles adhering and invading the host. Adhesins are responsible for adhering to other cells and therefore infecting them by attachment. These six proteins, the S protein and five non-structural proteins (nsp3, 3CL-pro, and nsp8-10) are the predicted vaccine targets (Ong et al. 2020). The S protein is a structural protein, and the nsp proteins are non-structural proteins. The S, nsp3, and nsp8 proteins were predicted as vaccine candidates due to their significant protegenicity scores. The protegenicity score reflects the compliment of proteins that are expressed by the SARS-CoV-2 virus. This score provides us with information that can help predict effective vaccine candidates. The Vaxign RV framework predicted that S protein would be the most favorable vaccine candidate due to it playing a role in the invasion of host cells and also because it had a high protective antigenicity score. Therefore, these factors confirm that the S protein is an important target for COVID-19 vaccines, but only targeting the S protein may not provide complete protection.

Because other proteins such as the nsp3 protein were deemed as possible targets for vaccines, they could also contribute to the production of a safe and effective vaccine. The nsp3 protein had the second highest protegenicity score and has the potential to contribute to induce complete protection from SARS-CoV-2. Due to nsp3's possible contribution to the vaccine, an "Sp/Nsp cocktail vaccine" is proposed as a result of these findings. Nsp3's high protegenicity score can be observed in Table 1. This table shows a variety of proteins with their Vaxign-ML score and their adhesin probability. A cocktail vaccine includes more than one antigen to cover different aspects of protection from the virus (Ong et al. 2020). This vaccine cocktail would include a structural protein (S protein) and a non-structural protein (e.g., nsp3). Having a mixture of protein antigens in the vaccine could induce more favorable protective immune responses than vaccines with only a structural protein. The production and the continuation of this research would need to be carried out before the use of a cocktail vaccine could be administered. Information regarding a vaccine for COVID-19 is still developing and will continue to evolve. It can take a long time to make a safe and effective vaccine and then distribute it to the masses.

Conclusion

COVID-19 is a deadly virus that has changed the way that we live and the way that we interact with others daily. COVID-19 is a similar virus to that of MERS-CoV and also SARS-CoV. The relationship of these viruses takes root in the shape of the virus itself. All three of these viruses are coronaviruses and mainly impact the respiratory system. This trend has been observed in humans and animals.

The use of animal models helped determine the susceptibility and transmissibility of the SARS-CoV-2 virus in animals and therefore helped determine how these factors could potentially affect humans. Results from this study concluded that both the CTan-H strain and the F13-E strain both exhibited replication in the respiratory system on cats and ferrets. These animals were chosen to be highlighted from the experiment because these animals provided the most conclusive evidence. Based on the findings of the animal model study, the CTan-H strain and the F13-E strain both replicated in the respiratory system of the animal models and respiratory droplet transmission occurred between one pair of cats. The transmission of the CTan-H strain was an important discovery because this is important to understand the transmission of the disease for humans as well.

There is still developing information about COVID-19, especially regarding the development of a vaccine and also the mental toll that this situation has on individuals. A vaccine has not been released to the public as of November 24, 2020. Currently, both Pfizer and Moderna are two companies that are working on getting approval for vaccine distribution. These companies are in the late stages of the development of a vaccine and each of the vaccines are predicted to be more than 90% effective. The impact of the pandemic on our mental health overall is negative, but there has not been conclusive data highlighting the mental state of individuals because it is vital to focus on physical health at this moment in time. It is important to note that this topic is presently under study and developing information that may not have been included in this research study. There are still many unknown aspects of COVID-19 that are subject to change. Overall, the use of animal models, past coronavirus outbreaks, and current vaccine technology will all contribute to overcoming the SARS-CoV-2 virus. Figure 5 provides a summary of the studies that were examined and provides connection among all of the different aspects that make up the bigger picture of SARS-CoV-2.

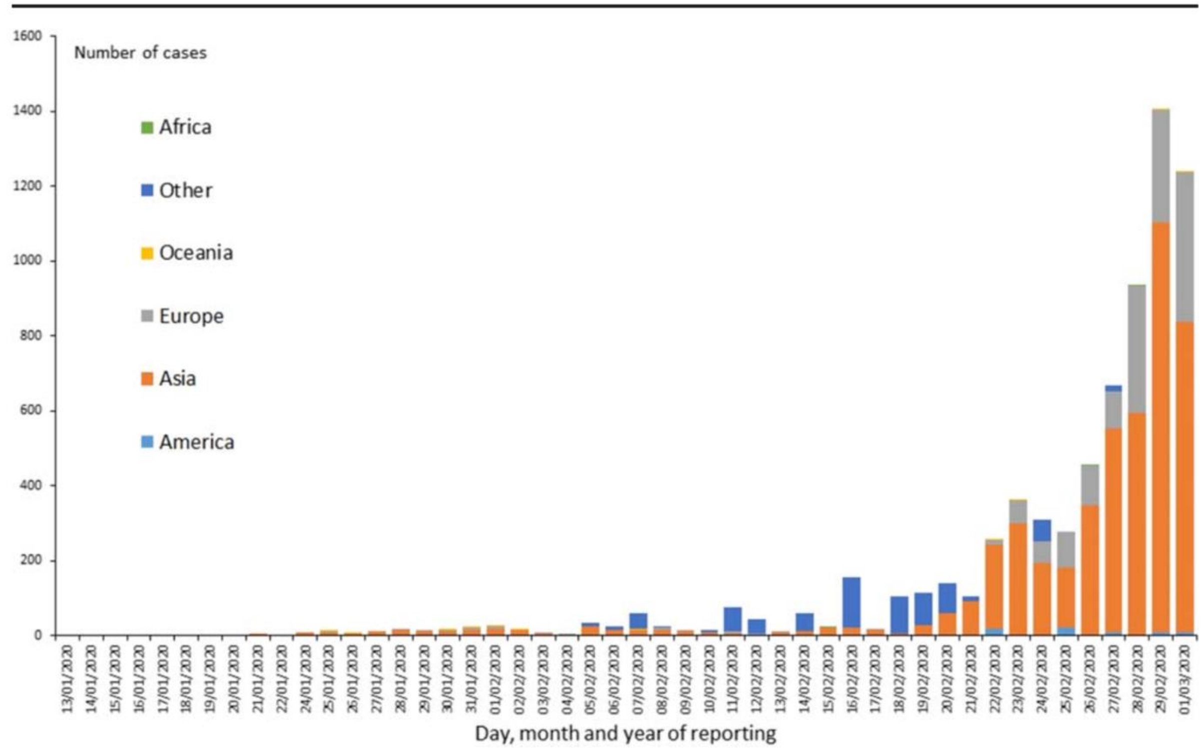

Figure 1. This figure shows the rapid increase in the number of cases of COVID-19 in different continents. The y-axis indicates the number of cases, while the x-axis shows the day, month, and year that the cases were reported, and the different continents that were analyzed are represented by different colors on the graph. This shows the sudden and rapid increase in reported cases in many continents. This graph was made from data of reported cases as of March 1, 2020. Image from Mohammadi et al. (2020).

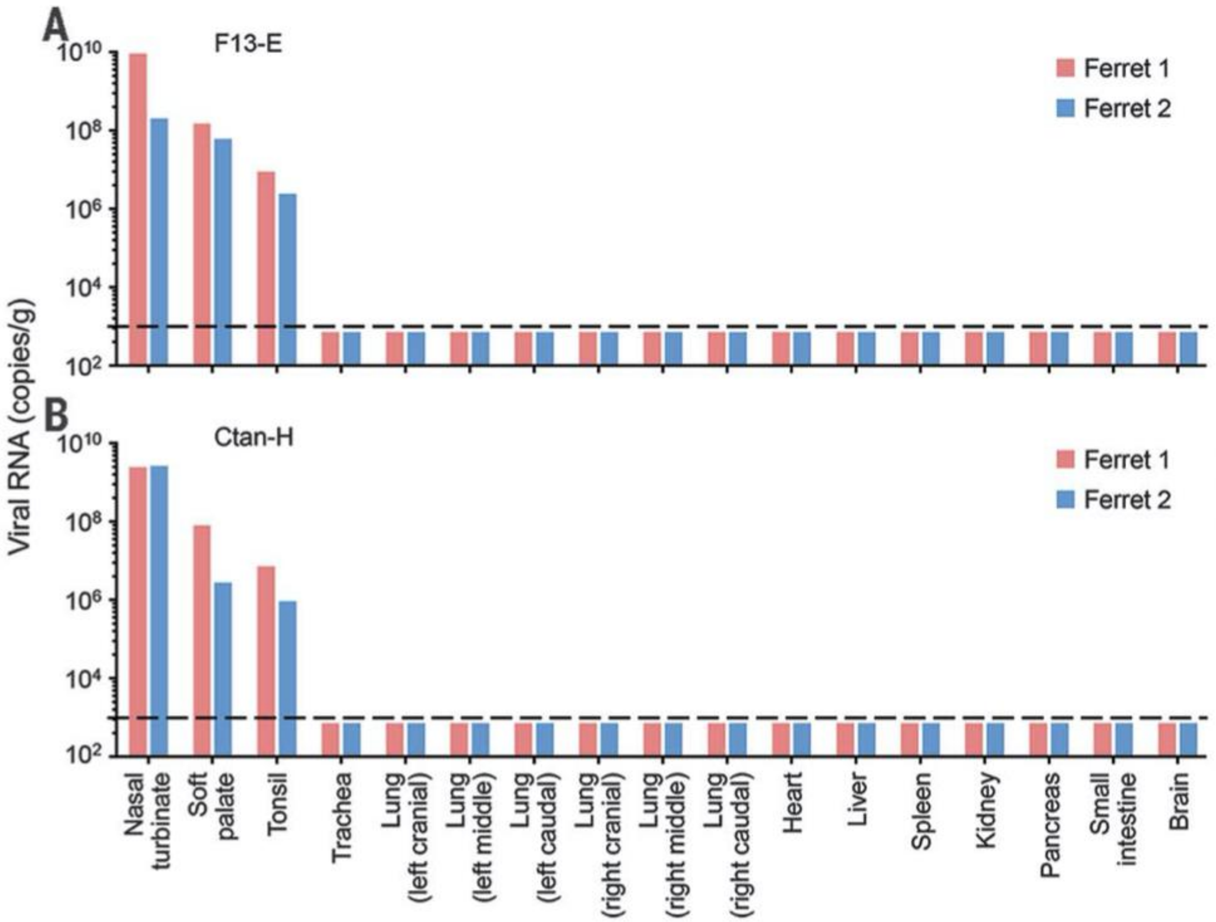

Figure 2. This graph shows the replication of the CTan-H strain and the replication of the F13-E strain in ferrets. The y-axis shows how much each strain replicated from an original inoculated amount of 10^5 PFU of each strain. This graph shows that each of the strains is replicated considerably in the upper respiratory system. Each strain is replicated specifically in the nasal turbinate, soft palate, and tonsils. This graph is modified from Shi et al. (2020).

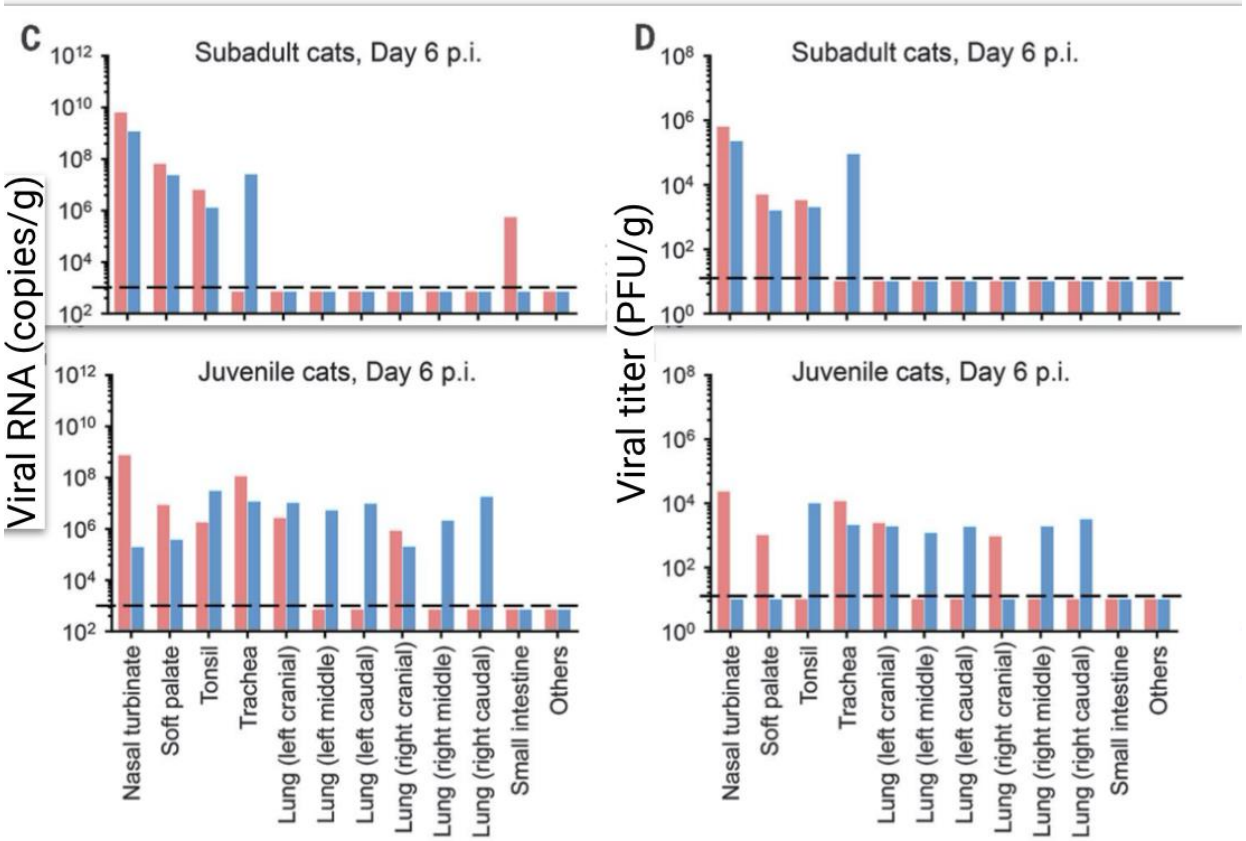

Figure 3. This graph shows the difference between the replication of the CTan-H virus in subadult and juvenile cats. The difference in the abundance and areas where the virus was detected in the two different age groups of cats can be attributed to a difference in age. The juvenile (younger) cats had a greater replication of the CTan-H strain and it was detected in more areas than the subadult (older) cats. This is different than what has been observed in humans, it has been observed that older individuals are more affected by SARS-CoV-2 than younger individuals. This graph was modified from Shi et al. (2020) to effectively show conclusive information.

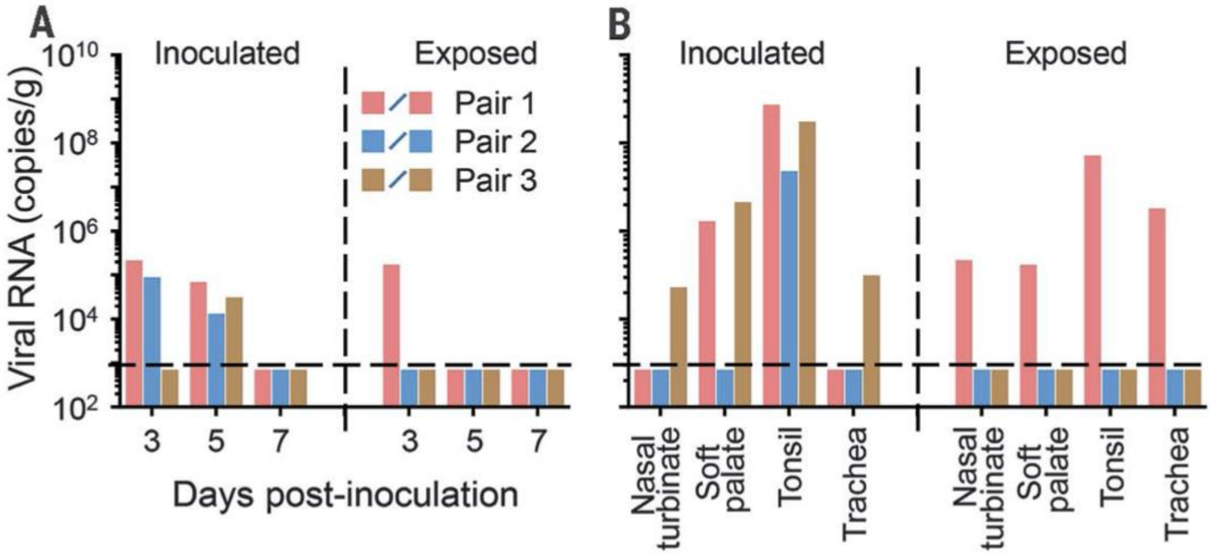

Figure 4. This graph is a visual representation of the analysis of respiratory droplet transmission between subadult cats. In this part of the experiment, three pairs of cats were examined. One individual cat from each pair of cats was inoculated with 10^5 PFU of the CTan-H strain. The other individual of the pair of cats was exposed to their inoculated pair. The cats were placed in adjacent enclosures in which they could not physically touch, but the air could travel between the enclosures. The left portion of the graph shows the amount of replication that occurred in each pair of subadult cats. The right portion of the graph analyzes respiratory droplet transmission of one pair of cats. This graph shows that respiratory droplet transmission occurred, and the virus replicated in the upper respiratory system. This graph was modified from Shi et al. (2020).

Table 1. This table shows the examined proteins and their correlating Vaxign-ML score and also their adhesin probability. These factors determine whether they would be good contributions to a possible cocktail vaccine. The S protein has the highest Vaxign-ML score, nsp3 has the second-highest and nsp8 has the third-highest score. In the study, it was suggested that the S protein and the nsp3 protein could possibly make an effective cocktail vaccine due to their high Vaxign-ML score. This table is from Ong et al. 2020.

TABLE 3 | Vaxign-ML prediction and adhesin probability of all SARS-CoV-2 proteins.

		Protein	**Vaxign-ML score**	**Adhesin probability**
orf1ab	nsp1	Host translation inhibitor	79.312	0.297
	nsp2	Non-structural protein 2	89.647	0.319
	nsp3	Non-structural protein 3	**95.283***	**0.524#**
	nsp4	Non-structural protein 4	89.647	0.289
	3CL-PRO	Proteinase 3CL-PRO	89.647	**0.653#**
	nsp6	Non-structural protein 6	89.017	0.320
	nsp7	Non-structural protein 7	89.647	0.269
	nsp8	Non-structural protein 8	**90.349***	**0.764#**
	nsp9	Non-structural protein 9	89.647	**0.796#**
	nsp10	Non-structural protein 10	89.647	**0.769#**
	RdRp	RNA-directed RNA polymerase	89.647	0.229
	Hel	Helicase	89.647	0.398
	ExoN	Guanine-N7 methyltransferase	89.629	0.183
	NendoU	Uridylate-specific endoribonuclease	89.647	0.254
	2′-O-MT	2′-O-methyltransferase	89.647	0.421
	S	Surface glycoprotein	**97.623***	**0.635#**
	ORF3a	ORF3a	66.925	0.383
	E	Envelope protein	23.839	0.234
	M	Membrane glycoprotein	84.102	0.282
	ORF6	ORF6	33.165	0.095
	ORF7	ORF7a	11.199	0.451
	ORF8	ORF8	31.023	0.311
	N	Nucleocapsid phosphoprotein	89.647	0.373
	ORF10	ORF10	6.266	0.0

Denotes Vaxign-ML predicted vaccine candidate.

#*Denotes predicted adhesin. Bold value denotes Vaxign-ML predicted vaccine candidate and/or predicted adhesin.*

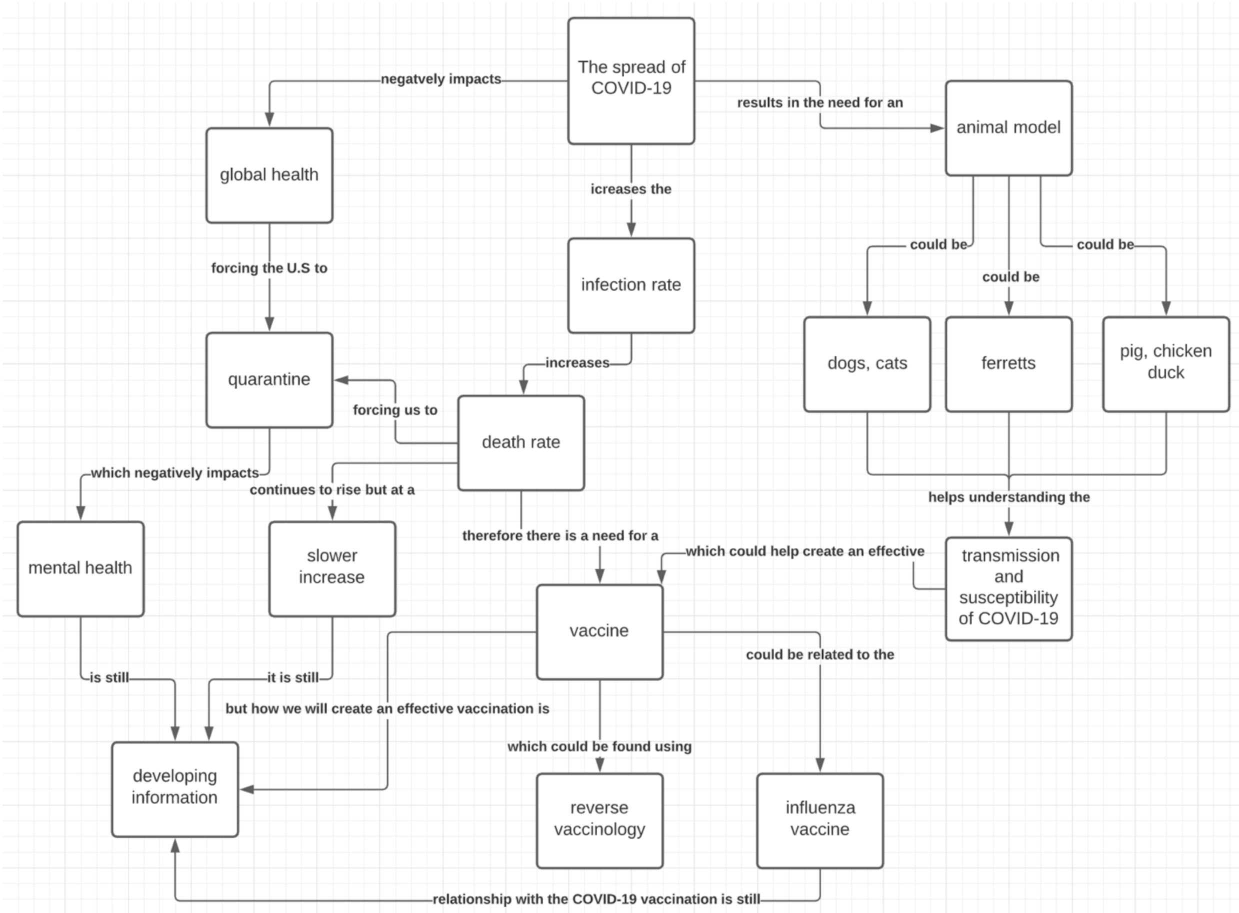

Figure 5. This figure is a visual representation of the underlying factors that have been included in this research analysis of COVID-19. It is important to understand the interrelatedness of these factors and how they connect to the overarching topic of SARS-CoV-2.

Works Cited

CDC. 2019 Nov 15. Basics of Vaccines. Centers for Disease Control and Prevention. [accessed 2020 Oct 25]. https://www.cdc.gov/vaccines/vpd/vpd-vac-basics.html.

CDC. 2020 Sep 11. Coronavirus Disease 2019 (COVID-19) – Prevention & Treatment. Centers for Disease Control and Prevention. [accessed 2020 Oct 17]. https://www.cdc.gov/coronavirus/2019-ncov/prevent-getting-sick/prevention.html.

Harrison EA, Wu JW. 2020. Vaccine confidence in the time of COVID-19. European Journal of Epidemiology. 35(4):325–330. doi:10.1007/s10654-020-00634-3.

Lakdawala SS, Menachery VD. 2020. The search for a COVID-19 animal model. Science. 368(6494):942–943. doi:10.1126/science.abc6141.

Maunder RG. 2009. Was SARS a mental health catastrophe? Gen Hosp Psychiatry. 31(4):316–317. doi:10.1016/j.genhosppsych.2009.04.004.

Mohammadi M, Meskini M, do Nascimento Pinto AL. 2020 Apr 19. 2019 Novel coronavirus (COVID-19) overview. Zeitschrift fur Gesundheitswissenschaften = Journal of Public Health.:1–9. doi:10.1007/s10389-020-01258-3.

Ong E, Wong MU, Huffman A, He Y. 2020. COVID-19 Coronavirus Vaccine Design Using Reverse Vaccinology and Machine Learning. Frontiers in Immunology. 11:1581. doi:10.3389/fimmu.2020.01581.

Ruiz SI, Zumbrun EE, Nalca A. 2017. Animal Models of Human Viral Diseases. Animal Models for the Study of Human Disease.:853–901. doi:10.1016/B978-0-12-809468-6.00033-4.

Shi J, Wen Z, Zhong G, Yang H, Wang C, Huang B, Liu R, He X, Shuai L, Sun Z, et al. 2020. Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS–coronavirus 2. Science. 368(6494):1016–1020. doi:10.1126/science.abb7015.

Torales J, O'Higgins M, Castaldelli-Maia JM, Ventriglio A. 2020. The outbreak of COVID-19 coronavirus and its impact on global mental health. The International Journal of Social Psychiatry. 66(4):317–320. doi:10.1177/0020764020915212.

Zoonotic Diseases | One Health | CDC. 2020 Feb 19. [accessed 2020 Nov 3]. https://www.cdc.gov/onehealth/basics/zoonotic-diseases.html.