The Efficacy of COVID-19 Detection in *Canis lupus familiaris*

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I. Abstract

The purpose of this study was to evaluate the efficacy of trained scent detection dogs (*Canis lupus familiaris*) to identify SARS CoV-2 (COVID-19) and emerging variants from specific human scent imprints. The efficacy of scent detection dog testing was compared to the Reverse Transcription Polymerase Chain Reaction (RT-PCR) method and qualitatively examined through various published studies conducted throughout the United States. SARS-CoV-2 shows rapid evolutionary rates; therefore, peer-reviewed literature was assessed to determine detection dog capacity to identify new coronavirus variants. Scent detection dogs exhibit great promise of becoming a reliable and immediate COVID-19 testing method, which empirical research demonstrated can be implemented at settings of mass gatherings. The second research method implemented was primary data collection, through the administration of a survey. The survey objective was to collect data on the comfort level of a random U.S. population sample regarding COVID-19 scent detection dogs compared with the at-home testing kit and RT-PCR testing efficacy. The survey was administered to 450 adult participants (limited to those 18 years of age and older) using the for-research survey distribution platform Prolific (https://www.prolific.co/). Prolific distributed the survey nationwide, resulting in the best opportunity for an unbiased participant population. The 450 participants were representative of a cross-section of the U.S. demographic. Principal Components Analysis (PCA), Correlation Analysis (CA), and UPGMA Cluster Analysis were applied to examine statistical properties among the 450 respondents based on 23 variables. Results indicated a variety of opinions regarding the employment of COVID-19 scent detection dogs and the reliability of the different COVID-19 testing method types. Overall, this study suggested a lack of knowledge and education in the sample population regarding COVID-19 scent detection dogs. With proper education about the current research, the survey

results suggest there may be public support for (COVID-19) detection dog implementation worldwide.

II. Introduction

Background

SARS CoV-2 (COVID-19/coronavirus) was first identified in December 2019 in Wuhan, China and has since notably impacted humans worldwide (Centers for Disease Control and Prevention [CDC], 2020). In March 2022, two years since the World Health Organization (WHO) officially determined COVID-19 a pandemic, people throughout the world, in developed and underdeveloped countries, continue to suffer the health and economic impacts of this virus. The COVID-19 pandemic emphasizes the importance of rapid and dependable testing to effectively slow the spread of this and any pathogenic infections in the most rapid and efficacious manner possible. Because the coronavirus is highly infectious, e.g. transmissibility and pathogenicity, exhibits rapid evolutionary rates via mutations, and presents asymptomatically, the CDC recommends the following to slow viral spread: extensive testing and contact tracing, approved face coverings, and social distancing. Current U.S. conditions (March 2022) demonstrate these practices are far more effective to slow and eventually stop the outbreak earlier rather than later. In addition, the unvaccinated population is at high risk and as the virus has available hosts, it has the potential to evolve into new viral variants, e.g. Delta, Mu, and Omicron (CDC, 2020; CDC, 2021; CDC 2022).

As new SARS-CoV-2 variants emerge, the most prevalent problem observed is increased transmissibility compared with the original variant (Van Oosterhout et al. 2021). The continued viral evolution makes controlling SARS-CoV-2 even more challenging (Van Oosterhout et al. 2021). R_0 is how the rate of transmissibility is measured and reported (Achaiah et al. 2020). The

first COVID-19 variant R_0 was estimated between $R_0 = 1.4$ and 2.4 by the WHO (Achaiah et al. 2020). $R_0 = 1.0$ is required for a virus to be transmissible between/among individuals (Achaiah et al. 2020). As each new COVID-19 variant evolved, the R_0 value increased, explaining the increased number of infected individuals (Achaiah et al. 2020). These findings also demonstrated the desperate need for rapid and reliable testing methods for COVID-19 worldwide.

The most prominent methods currently employed for COVID-19 detection are dependent on Reverse Transcription Polymerase Chain Reaction (RT-PCR). Figure 1 represents RT-PCR based on the well-known TaqMan hydrolysis probes (Yüce et al. 2021). These approaches are known as viral tests, which generate results indicating if an individual is positive for the COVID-19 virus. Other testing methods include antibody tests, most referred to as serology tests, where blood samples are collected. These tests show if an individual was positive in the past, but failed to confirm current viral infection (Yüce et al. 2021). Wiersinga et al. (2020) reported both RT-PCR viral detection and serum antibody testing methods displayed problems and uncertainties in the outcome. Essler et al. (2021) showed false negatives resulted from challenges with sampling and false positives might result from the presence of viral RNA without replication-competent virus.

In developing countries, RT-PCR results are often time-consuming and cost-prohibitive, where supplies are limited and finances must be channeled elsewhere; therefore, testing has generally been directed towards those individuals experiencing COVID-19 symptoms. Due to these restrictions, it is clear a faster, more reliable, and cost-effective method for COVID-19 testing is necessary. In addition, the method should be readily available for all, not just those

suspected of suffering from COVID-19. Finally, one consequence of a lack of testing includes SARS-CoV-2 evolution.

The successful use of detection dogs in olfactory-based tasks is attributed to multiple characteristics and advantages, both anatomically and physiologically (Singletary et al. 2021). Canines possess specialized olfactory neuroepithelium, which covers a large surface area and is densely packed with olfactory receptor cells, collectively expressing approximately 825 different odorant receptors (Singletary et al. 2021). Another advantage of the canine olfactory system is a specialized olfactory recess directly destined for the olfactory neuroepithelia, where the odors are recognized, which likely contributes to the increased olfactory acuity in dogs (Singletary et al. 2021). This recess receives upwards of 20% of the inspired air, whereas active sniffing is estimated to direct about 2.5 times more air than would be possible without the presence of the recess, to the olfactory recess per unit of time (Singletary et al. 2021). The complexity of a canine's anatomical olfactory system (Fig. 2, Aguiar 2019) serves to substantially increase the total surface area of the receptive field, measured at more than 200 cm^2 and provides a notable advantage in their sense of smell and odorant detection capabilities (Singletary et al. 2021).

Ensminger (2011) described the decades-long use of scent dog detection in various applications, including, but not limited to, 'sniffing out' materials such as firearms, explosives, and prohibited drugs. Other notable specialized fields for detection dogs include, pests, biological threats, conservation, ecology, agriculture, human remains, etc. (Singletary et al. 2021). However, more recently the medical field discovered canine olfactory perception an incredibly useful tool. Several studies found *C. lupus familiaris* possessed the ability to detect the presence of infectious and non-infectious diseases (Jendrny et al. 2020), including malaria (Guest

et al. 2019), several types of cancer (McCulloch et al. 2006), and numerous viral and bacterial infections (Taylor et al. 2018; Angle et al. 2016a; Angle et al. 2016).

Consequently, it was hypothesized, based on information already known about scent dogs' detection capacity to identify certain diseases, *C. lupus familiaris* might successfully detect the presence of COVID-19 by 'sniffing' specific human SARS-CoV-2 imprints. This hypothesis led to COVID-19 scent detection dog training, laboratory-based research, and successful testing implemented in public forums, i.e. at the site of large or mass gatherings, such as airports and sporting events. In this way, immediate results are generated, preventing further coronavirus spread (Jendrny et al. 2020; Wiersinga et al. 2020).

Research Null Hypotheses

Ho_1: Scent detection dogs (*Canis lupus familiaris*) show equal if not greater efficacy in detecting COVID-19 on human subjects compared with commonly used methods, such as RT-PCR.

Ho_2: Canine olfactory senses successfully learn to detect the rapidly evolving COVID-19 viral variants and are more efficacious than laboratory methods.

Ho_3: The majority of the survey participants will rank RT-PCR testing more reliable than scent dog detection for COVID-19.

III. Methodology

Literature Search

A literature search was conducted to find all published works in the primary literature that report the olfactory efficacy of scent detection dogs to successfully identify the presence of SARS-CoV-2 in human individuals. A primary literature review from empirical studies was conducted to qualitatively compare COVID-19 scent dog detection to the Reverse Transcription

Polymerase Chain Reaction (RT-PCR) testing method. Supporting primary literature was also

examined to review the history of *C. lupus familiaris* in detecting other infectious diseases and

scent detection dogs' capability to detect new coronavirus variants as the virus rapidly evolves,

e.g., Delta, Mu, and Omicron BA.1 and BA.2. Primary peer reviewed scientific journal articles

and scientific study reports were included in the search. I used the following databases and

search terms, respectively: https://scholar.google.com/ , worldwidescience.org, researchgate.net,

PubMed (https://www.ncbi.nlm.nih.gov/pmc/), and Web of Science (clarivate.com); scent dog

detection of COVID-19/ SARS CoV-2; RT-PCR COVID-19 testing method; scent dog detection

of diseases or illnesses; and canine olfactory sense. Articles included in the present study were

those reporting empirical studies, which included data on the olfactory efficacy of scent detection

dogs to sense the presence of SARS-CoV-2 in human individuals compared to at-home testing

kits and RT-PCR testing methods. A qualitative assessment was conducted regarding the

historical viewpoint of scent detection dogs in the medical field, the effectiveness of scent

detection dogs in identifying COVID-19, and their expected efficacy and capability in adapting

to the evolving nature of SARS-CoV-2.

Survey Literature Search

The literature review served to provide background to establish the basis for the survey

variables. The knowledge gained by studying how scent detection dogs are trained, how they

alert positive cases of COVID-19, their success and accuracy rates, and how researchers predict

future employment of scent detection dogs was vital to formulating an effective and complete

survey. The new information researchers learned from conducting their studies served to guide

the types of questions and variables appropriate for the questionnaire. Similarly, some of the

limitations expressed by the researchers also raised questions that were explored through the

survey. For example, various research teams noted one of the greatest concerns of implementing COVID-19 detection dogs was the lack of government support and funding and the population's comfort level with dogs. Therefore, the survey included these questions and others derived from the current body of literature.

Survey Data Collection

The survey objective was to collect data from a cross-section of the US demographic using 23 variables (Appendix XI). The survey was a random sample of participants' (N=450) responses on the efficacy of scent detection dogs' ability to identify the coronavirus compared with other testing methods as well each participant's experiences with COVID-19. Before administering the survey, Human Studies Approval from the Office of Grants and Research Development (IRB) was obtained.

The survey was administered to 450 adult participants (18 years of age and older) using the for-research survey distribution platform Prolific (https://www.prolific.com/). Prolific distributed the survey nationwide, resulting in a geographically unbiased population of participants. Prolific gives the option for prescreening criteria that must be met by the participants. Using Prolific to distribute the survey resulted in a population sample that reflects the demographic distribution of the United States.

Once the 450 survey responses were collected, various statistical methods were conducted to analyze the data. Principal Component Analysis (PCA) was performed to reduce the dimensionality of the larger dataset while preserving relevant statistical information, such as correlations among data points (Jolliffe and Cadima 2016). First, a scree plot (Fig. 4) determines whether a PCA is compatible with the data set. Essentially, if most of the variation in a data set can be captured within the first three principal components, a PCA is a suitable statistical tool.

Principal Component 1 (PC1) represents the maximum variability between the data with minimum error and each subsequent PC (PC2 and PC3 in the analysis) represents a slightly lesser degree of variation. A biplot (PCA loading plot + PCA score plot) (Fig. 5, 6) is constructed to display clusters of samples based on their similarity (obtained from PCA score plot) and how strongly each characteristic influences a certain PC (obtained from PCA loading plot) (Team 2018). Each individual represented a single point in the biplot and each variable represents a vector. Data points clustered around a particular vector are likely influenced by the corresponding variable, and the further the vector is from the origin, the stronger the relationship. Further, vectors that create angles of less than 90 degrees are likely to be positively correlated. Conversely, vectors that form angels larger than 90 degrees are likely to be negatively correlated with one another and vectors creating angles of 90 degrees are unlikely to be correlated. The UPGMA Cluster Analysis is a distance analysis method that clusters variables together based on similar relationships among the respondents (Weiß and Göker 2011). These clusters are expected to be similar to those provided by the PCA biplots, and therefore, should show consistency among the data. The strength of the relationship between a cluster is signified by the distance a node is away from the axis, i.e. the closer the node, the stronger the relationship (Fig. 8). Pearson's Correlation Analysis (CA) provided a Pearson's correlation coefficient (r) that determined the strength and direction of any relationship found in the data between variables. The closer the *r-value*, the stronger the correlation between two variables, e.g. $r = 1$ indicates a perfect positive correlation and $r = -1$ indicates a perfect negative correlation. A positive correlation shows where respondents answered similarly to the two variables compared. The opposite is true of a negative correlation, where the respondents had a differing opinion to the two variables.

IV. Results

Literature Search Findings

Essler et al. (2021) proposed a potential solution to solve issues in current testing for COVID-19, implementing testing based on the volatile organic compound signature (VOC) of SARS-CoV-2 infections. Domestic dogs are biological sensors for several diseases with unique VOC profiles from different biological fluids, including urine and saliva (Essler et al. 2021). Essler et al. (2021) provided strong evidence of a unique VOC profile associated with SARS-CoV-2 infection, and *C. lupus familiaris* can be trained to recognize the human scent profile from saliva and sweat samples. If dogs are successfully and accurately able to discriminate VOCs in samples associated with SARS-COv-2 infection in samples from non-infected individuals, then this opens the door to novel screening methodologies based on VOC signatures including electronic noses (a device used to provide an easy and inexpensive way to analyze gas samples for medical diagnostics) and scent detection dogs (Essler et al. 2021). A VOC-based approach could result in nearly instant detection, where large numbers of people could be screened rapidly and if dogs are utilized, it might be possible to conduct screenings of large numbers of people noninvasively and without delay (Essler et al. 2021).

In most studies, dog training was completed using a device called Detection Dog Training System (DDTS) for dogs to become familiar with the SARS-CoV-2 scent (see video Appendix 2). This device presents samples in an automatic and randomized manner, without trainer interference (Jendrny et al. 2020). DDTS is composed of seven scent holes and two tubes are located behind each hole leading to two metal containers (Jendrny et al. 2020). Only one container is presented in each scent hole at any given time, and these containers are enclosed with grids, allowing the odor to escape and reach the scent hole (Jendrny et al. 2020). The dogs

are prevented from making physical contact with the samples due to the presence of identical L-shaped tube extensions (Jendrny et al. 2020). This design also excluded any visual cues that might provide additional detection capabilities (Jendrny et al. 2020).

The training procedure is as follows: "for each trial run, only one hole presented a SARS-CoV-2 positive sample at a time while the other six holes presented negative samples. After the indication of the hole with the positive sample, the dog was automatically rewarded by the device with food or ball. The indication time was changed during successful training from 1 s to 2 s. While the reward was eaten, the device's software randomly and automatically assigned new positions to the slides for the following session with again only one hole presenting the positive odor sample" (Jendrny et al. 2020). The entire training process is completed in a double-blind manner, where the dog, its handler, and a person observing the study were all unaware of the sample placement (Jendrny et al. 2020). All personnel stood behind the dog during the test runs to avoid distraction. The device automatically recorded the number and length of time of each nose dip into the scent hole, as well as the location of the positive and negative samples (Jendrny et al. 2020). The recorded data was subsequently verified by manual time-stamped video analysis after the test runs. Following a short two-week habituation process to the DDTS, the dogs required only five days of training until the detection rate exceeded results obtained by chance alone (Jendrny et al. 2020).

After the detection training, Jendrny et al. (2020) proceeded to collect research data. Results showed within randomized and automated 1012 sample presentations, the detection dogs achieved an overall average detection rate of 94% ($\pm$ 3.4%) with 157 correct positive indications, 792 correct negative rejections, 33 false positive and 30 false negative indications (Jendrny et al. 2020). The presented samples were a mixed collected of tracheobronchial and

saliva secretions. There was no statistically significant difference in detection ability between saliva and tracheal secretions (Jendrny et al. 2020). Based on these successful results, the researchers concluded that SARS-CoV-2 detection dogs have the ability to provide an effective and reliable infection detection technology in various settings, such as public facilities, and to function as an alternative or addition to regular RT-PCR screening (Jendrny et al. 2020).

One of the most preliminary studies of COVID-19 odor detection dogs was conducted by Florida International University (Mendel et al. 2021). The study explored and successfully demonstrated the use of *C. lupus familiaris* to detect COVID-19 through exhaled breath (Mendel et al. 2021). Originally, the research intended to use COVID-19 odor on contaminated surfaces (Mendel et al. 2021). As the initial research continued, it evolved into testing the dogs' abilities to detect COVID-19 odor on infected individuals (Mendel et al. 2021). The researchers used masks obtained from hospitalized patients that tested positive for COVID-19 (Mendel et al. 2021). Four dogs were trained and evaluated for their ability to accurately detect the virus (Mendel et al. 2021). The results indicated that all four dogs obtained an accuracy of greater than 90 percent and positive predictive values ranging from about 73 to 93 percent after only one month of training (Mendel et al. 2021).

A study performed at the University of Pennsylvania showed scent detection dogs were easily and successfully trained within eight weeks to detect SARS-CoV-2 (Gantz 2021). During their training, the detection dogs were never presented with a live version of the virus. This was so the individuals training the dogs were not exposed to COVID-19. Despite never coming across a live COVID-19 virus, these scent dogs were still able to detect live viruses within infected humans. Each of the nine trained dogs, with no prior experience with medical detection, exhibited a 96% accuracy when detecting individuals with COVID-19 (Gantz 2021).

One of the most concerning questions involved in this area of research and field-applications is if detection dogs' abilities can "adapt" to the rapid rate of COVID-19 evolution. As research commences on developing COVID-19 variants, scent detection dogs are very successful in identifying variants, including the delta, mu, and Omicron variants. Rodriguez (2021) reported in Hawaii, scent detection dogs detected the delta variant with up to 90% accuracy. Limm (2021), advocated for COVID-sniffing dogs to be implemented as a part of airport screening and at the entrance of highly populated sites, such as shopping malls. Limm (2021) also suggested this method of COVID-19 identification could swiftly identify pre-symptomatic and asymptomatic individuals, limiting the spread.

The empirical research shows scent detection dogs accurately identify COVID-19 in humans. Hag-Ali et al. (2021) subsequently applied Bayesian analysis to assess the sensitivity of scent dogs to detect COVID-19 and found *C. lupus familiaris* superior to RT-PCR testing. Hag-Ali et al. (2021) pooled the results from 16 different studies and found RT-PCR showed an estimated sensitivity to detect SARS-CoV-2 of 87.8%, although more recent evidence suggested the specificity of the test was moderate, with a sensitivity of 63%-78% (Hag-Ali et al. 2021). These sensitivity values indicated a positive test result was highly indicative of a COVID-19 infection, but a negative test does not completely rule out the virus (Hag-Ali et al. 2021).

In a comparative study, Hag-Ali et al. (2021) reported scent dog detection sensitivity (i.e., a test's ability to designate an individual with disease as positive) and specificity (i.e., the test's ability to designate an individual who does not have a disease as negative) were 83.3% and 99.2%, respectively. This study also calculated predictive values (i.e., the likelihood that a test can successfully identify whether individuals do or do not have a target condition, in this case COVID-19). The positive predictive value (the ability to identify positive COVID-19 results) of

detection dogs was 52%, reflecting the probability individuals with a positive screening test had Sars-CoV-2 (Hag-Ali et al. 2021). The negative predictive value (the ability to identify negative COVID-19 results) was 99.8%, indicating the probability individuals with a negative screening test did not have Sars-CoV-2 (Hag-Ali et al. 2021). Hag-Ali et al. (2021) reported scent dog detection overall accuracy was 99.1% and the proportion of individuals incorrectly labeled by a detection dog was 0.9%. Furthermore, the research reported the dog detection tests showed higher sensitivity than RT-PCR, but both methods exhibited high and similar specificity, i.e. 99% (Hag-Ali et al. 2021). The high specificity value of both testing approaches indicated notable utility in accurately identifying the truly negative COVID-19 individuals; however, scent dog detection tests are a quick and low-cost alternative to routine screening for COVID-19 and have low transmission risks in comparison with PCR during sample collection (Hag-Ali et al. 2021).

Bellware and Suliman (2021) reported locations and organizations where COVID-19 screening by disease detection dogs is already in place. For example, the Miami International Airport employs COVID-19 scent detection dogs for airport employees. At one of the nation's busiest airports, all employees are required to pass a 'sniff test' by one of the two seven-year-old dogs trained to detect the presence of the coronavirus. The Miami International Airport is the first American airport to recruit dogs in COVID-19 prevention and control strategies (Bellware and Suliman 2021). The two disease detection dogs spend their shifts sniffing the face coverings of employees passing through a checkpoint to detect the presence of the virus in sweat, breath, and scents due to metabolic changes the virus causes in the human body (Bellware and Suliman 2021). If one of the dogs signals the odor of the virus on a person, that individual is directed to

take a rapid COVID-19 test. Each individual dog's accuracy is reported at 98.1% and 99.4 %

(Bellware and Suliman 2021).

Tori (2022) described two Labrador Retrievers trained as a part of a study conducted by

Florida International University and transferred to Massachusetts, where the COVID-19 scent

detection dogs work at fifteen different schools, various police stations (for state emergency

operations), a local health department, town halls, and office buildings. The detection dogs are

trained to sit down in front of an individual where a COVID-19 odor is detected (Tori 2022).

These dogs can also detect an area where they detect a COVID-19 odor, alerting their handler so

they can mark the area for appropriate cleaning.

A hospital in Sarasota, Florida is utilizing disease detection dogs to help doctors identify

COVID-19 positive patients (Lane 2021). After all hospital visitors pass through the temperature

check and initial COVID-19 screening questions, a scent detection dog sniffs the visitor. If an

individual is detected with COVID-19, the detection dog lays down to alert the handler.

Following a positive COVID-19 detection, the individual is asked to take a rapid test. The

Sarasota, Florida hospital SARS-CoV-2 scent detection dog was shown to be 95% accurate at

identifying positive COVID-19 cases (Lane 2021). The hospital staff reported the dog has been a

beneficial extra level of protection used to make sure the patients and staff stay safe.

Survey Results

The survey was distributed through Prolific and resulted in 450 responses (N = 450).

Prolific bases its United States distribution on US Census data, so participants should be an

adequate representation of the US population. Results showed variability based on ethnicity, age,

sex, and education level (see Table 1).

Results of the Correlation Analysis (CA) (Fig. 3) indicated the strength and direction of correlations between variables (r-values) and which survey variables exhibited significant correlations based on participant responses. Table 2 shows significant correlations at the 0.05 a $priori$ level of significance. The highest significant correlations (r-values) were observed between the COVID-19 testing methods. A strong positive correlation ($r = 0.97$, $P < 0.05$) was detected between survey respondents who answered they were tested for COVID-19 (Cov Test) with RT-PCR by nasal swab (Nas test). In addition, a strong positive correlation ($r = 0.95$, $P < 0.05$) was observed between saliva sample COVID-19 tests (Sal test) and COVID-19 scent detection dogs (Dog test).

A positive moderate and significant correlation ($r = 0.62$, $P < 0.05$) was found for participants supporting government approval and funding for COVID-19 detection dogs (Gov. appvl) and support to implement COVID-19 detection dogs worldwide (Wrldwide) (Table 2). Another moderately positive and significant correlation ($r = 0.67$, $P < 0.05$) was observed between the validity of COVID-19 scent detection dogs to identify emerging variants (Emrg vrnt), e.g. Omicron BA.2, variants reported to evade vaccines, and detection dogs' reliability in future pandemics (Futr pan). A moderately negative and significant correlation (($r = -0.55$), $P < 0.05$) was observed between scent detection dogs' reliability to identify COVID-19 (Rel dog) and if scent detection dogs are a more viable testing approach for emerging COVID-19 variants (>viable). Interestingly, when compared to whether participants would still attend mass gathering events if COVID-19 detection dogs were employed to "sniff-out" positive cases (Atten), many variables resulted in significant weak positive correlations ($P < 0.05$), suggesting participants exhibited a wide range of responses.

The PCA Scree Plot supported the expectation that most the data variability was within the first three principal components, with the greatest level of variability observed at PC1 (Fig. 4). Continuing along the x-axis, the variability steadily leveled out at PC2. Figure 5 shows the first biplot comparing the first and second principal components. This biplot revealed two distinct clusters of variables. On the right side of the y-axis, the following variables were clustered: Ethn, Rel home, Rel dog, Cmft dog, Rel PCR, Age, and Edu. This group included Age and Ethn, and Cmft dog and Edu, two pairs of variables allied based on the variables and respondents. The cluster of variables on the right side of the y-axis included Rel home, Rel dog, and Rel PCR, which were allied based on variable attributes. On the left side of the y-axis, the following variables were clustered: >viable, Cov test, Emrg vrnt, Omi BA.2, Futr pan, Ctrl loc, Atten, Sex, Gov appvl, Wrldwide, Nas test, Sal test, Dog test, Pos Cov, Nas Pos, Sal Pos, and Dog Pos. This variable grouping comprised all COVID-19 testing methods and the responses for COVID-19 positive test results. The remaining variables (>viable, Cov test, Emrg vrnt, Omi BA.2, Futr pan, Ctrl loc, Atten, Sex, Gov appvl, Wrldwide) were closely allied based on responses of participants to the questionnaire.

A second biplot compared the first and the third principal components (Fig. 6). This biplot was indicative of the same two distinct clusters of variables. One notable difference between the two biplots was the location of the Cov test variable. In the PCA 1-2 biplot, Cov test was grouped with >viable, Cov test, Emrg vrnt, Omi BA.2, Futr pan, Ctrl loc, Atten, Sex, Gov appvl, and Wrldwide. In the PCA 1-3 biplot, Cov test was more appropriately clustered with the COVID-19 testing methods and the reported COVID-19 test results. It must be emphasized these data are distributed in 3-D, so the interpretation of PCs 1 vs. 2 and 1 vs. 3 generate a more accurate depiction of the patterns of variability and relationships among variables based on

participant responses. Figure 7 provides a 3-D scatter plot, illustrating these results three dimensionally.

Survey results showed UPGMA variables clustered into the same two groups observed from PCA (Fig. 8). The first cluster represented survey response similarities with the following variables: >viable, Cov test, Emrg vrnt, Omi BA.2, Futr pan, Ctrl loc, Atten, Sex, Gov appvl, Wrldwide, Nas test, Sal test, Dog test, Pos Cov, Nas Pos, Sal Pos, and Dog Pos. This UPGMA cluster suggested the participants answered most similarly in their opinions for Emrg vrnt, Omi BA.2, and Futr pan. Close similarities were also observed between Gov appvl and Wrldwide, and all COVID-19 testing questions. The second cluster suggested survey response similarities for the following variables: Ethn, Rel home, Rel dog, Cmft dog, Rel PCR, Age, and Edu. The second cluster indicated the participants answered most similarly regarding their comfort level with dogs, the reliability of PCR testing for COVID-19, the reliability of home COVID-19 tests, and reliability of COVID-19 detection via scent detection dogs. Age and education level were the furthest removed, most notably education, suggesting high variability in these variables.

V. Discussion

Economics of Disease: Detection Dogs

The literature provided clear evidence that *Canis lupus familiaris* are highly successful at detecting COVID-19 (Essler et al. 2021; Gantz 2021; Hag-ali et al. 2021; Jendrny et al. 2020; Lane 2021; Mendel ett al. 2021). Consequently, it is curious why this highly effective approach to SARS-CoV-2 detection in humans is not implemented nation- or worldwide. One potential deterrent is economics. The cost of disease detection dogs varies substantially. One estimate from the Transportation Security Administration (TSA) reported start-up costs to train bomb-sniffing dogs at over $200,000 and an additional $150,000 after accounting for the cost of the

handler, veterinary care, food, and certification (Otto et al. 2021). Otto et al. (2021) provided an additional TSA estimate for training a screening dog and handler of $42,000. In addition, some estimates declared a price for acquiring a dog at $10,000 and $16,000 for scent dog detection school.

While it is essential to note the starting costs, it is also important to evaluate what these funds will provide. Estimating a scent detection dog can sniff 250 people per hour, working a six-hour day for 30-minutes on, 30-minutes off, five-days per week, a single dog can potentially screen > 189,000 people in a year (Otto et al. 2021). Therefore, the cost per person to screen could range from 13.7 cents (for training and dog acquisition) to $1.05 (for a fully funded start-up) (Otto et al. 2021). Although there is a considerable amount of "sticker shock", the costs are approximated at $200 million, when considering the full start-up based on one thousand dog/handler teams, similar to what is already used by TSA for airports, mass transit, and maritime systems, and expenditures would likely decline based on economies of scale (Otto et al. 2021). Consequently, once a dog is trained to detect one scent, it has the potential to be trained to detect others, increasing utility and cost-effectiveness (Otto et al. 2021).

Hag-Ali et al. (2021) compared the cost-effectiveness of scent dog detection to the widely used RT-PCR for COVID-19 and found scent dog detection more effective. RT-PCR is costly, requires trained staff, expensive equipment, and reagents. Furthermore, given its high sensitivity, short turn-around-time, low cost, less invasiveness, and ease of application, detection dog tests appear a better alternative to RT-PCR in screening for SARS-CoV-2 in asymptomatic individuals (Hag-Ali et al. 2021). RT-PCR testing has a long turn-around-time, which hampers its use for rapid and long-term mass screening. The high negative predictive value of the scent detection dogs' tests supports the use for screening purposes, as it will determine individuals

who do not need the more expensive, time-consuming, and labor-intensive RT-PCR test (Hag-Ali et al. 2021).

Scent Detection Dogs: Appropriate Settings

Scent detection dogs have already shown great promise in COVID-19 identification. Consequently, these highly trained dogs will have value to public health across various settings with high risk of serious outcomes from the disease, such as very densely populated environments and mass gatherings. Otto et al. (2021) emphasized the notably high disease risk in settings such as long-term care facilities, where rapid screening of residents and staff is vital, as witnessed during the initial COVID-19 outbreak. High transmission risk is common among specific settings, such as prisons, food processing and/or manufacturing plants, and these environments show little leverage to reduce population densities (Otto et al. 2021). Other key settings include supporting the operation of essential services, such as public transport, police, fire, health, and education (Otto et al. 2021).

Otto et al. (2021) reported it is judicious to implement detection dogs in largely congregated settings, where many people must be screened in a short period of time and in a physical environment where people routinely move through central checkpoints. A few examples include, but are not limited to, airports, K-12 schools, concert halls, and sporting events. These are the types of environments where individuals are directed to move through a location in an orderly manner, so adding detection dogs to this process could easily be executed (Otto et al. 2021). It is important to note this type of COVID-19 detection would supply the information needed to indicate where follow-up testing is needed and those noted as positive during screening would be directed to definitive testing services (Otto et al. 2021).

Limitations

Canis lupus familiaris clearly demonstrated an ability to differentiate between positive and negative SARS-CoV-2 samples; however, one study demonstrated a number of very important limitations (Essler et al. 2021). Essler et al. (2021) admitted that their study used repeated presentations of the same COVID-19 samples, so it is possible the result of a 94% accuracy rate was simply that the dogs were able to discriminate between their training set of positive and negative patient samples but were unable to generalize this odor to new samples. Essler et al. (2021) reported in the initial introduction of completely novel SARS-CoV-2 samples to scent detection dogs, the samples did not appear to pose a problem. However, after a period of intensive training on a limited number of new, random samples, the dogs appeared to move from generalization strategies to more discrimination of individual samples in the training set (Essler et al. 2021). Despite mixing the samples to increase the number of sample odor profiles to support their generalization of COVID-19 odor profiles for positive samples, Essler et al. (2021) were only able to document that the dogs generalized to novel known COVID-19 positive and negative sample mixes and were unable to generalize to completely novel samples (Essler et al. 2021). It was possible reducing the number of presentations the dogs had on identical training odors might have facilitated their generalization, but further investigation would have to be performed to draw this conclusion (Essler et al. 2021).

The results provided by Essler et al. (2021) raised important questions about training *C. lupus familiaris* to recognize a COVID-19 odor profile. The ideal sample number, the diversity of patients providing samples, and the extent of training required to generalize to novel samples in this odor problem remained unknown (Essler et al. 2021). Essler et al. (2021) found the training executed in this study did not result in documented generalizations of a SARS-CoV-2 positive odor profile, despite dogs showing impressive discrimination between positive and

negative samples. Therefore, these results suggested either the sample or presentation numbers must be better suited for discrimination and generalization equally (Essler et al. 2021). It was recommended future scent detection dog training and investigation into biological, chemical, and electronic detectors should focus on increasing the relevant and novel sample number. The more novel profiles of positive and confirmed negative samples without replication of individual patients will promote generalization by the disease detection dogs and identification by the sensors of the specific COVID-19 target odor profile (Essler et al. 2021).

Future Research

Although disease detection dogs are showing impeccable promise in detecting COVID-19, further research must be completed to ensure the dogs are able to indicate disease across large numbers of people. Otto et al. (2021) expressed some concerns that when dogs are placed in real-world settings, the power of their noses will be put to a true test. There might be factors with the potential to negatively influence the dogs' ability to detect disease, such as noise, air flow, temperature, humidity, ambient odors, and disease prevalence (Otto et al. 2021). The researchers suggest the following next steps to move forward with this testing method: (1) support for research to understand the potential and limitations of disease detection dogs during any pandemic response, (i.e. current, future, and when COVID-19 is classified an endemic); (2) establishment of standards for training and sentinel training sites; (3) identification of optimal operational sites for the use of disease detection dogs; and (4) show return on investment in other settings by using scent detection dogs for other diseases and establishing protocols for future pandemic use (Otto et al. 2021). Ultimately, disease detection dogs could be the future of COVID-19 testing and that of future pandemics, but more research is needed, as well as government and community investment to best move forward.

Discussion: Survey Results

The demographics (Table 1) chosen as part of the survey data (i.e., sex, age, ethnicity, and education) played a vital role in interpretation of results. Due to potential relationships between demographics and survey variables, (e.g. Cmft dog, COVID-19 testing reliability), it was necessary to include these types of data within the survey. It was also vital to inquire about the type(s) of COVID-19 testing each participant experienced, their ranking of each testing method, and which of the test(s) resulted in a positive COVID-19 result, if any, to best determine if the participants possessed any predisposed opinion(s) regarding a certain testing method (Appendix 1-Survey). The survey also served to determine respondents' views of the reliability of scent detection dogs, as this was one primary objective of the survey. The participants were asked to rank their comfort levels with dogs, once again to better understand where certain reservations might exist in the process to implement COVID-19 scent detection dogs worldwide as a reliable COVID-19 testing method. All variables resulted in a crafted survey, providing as many avenues to the subject as possible to effectively explore the opinions of the participant population.

CA (Table 2; Fig. 3) suggested that most significant correlations ($P < 0.05$) were moderate in strength. These results overall indicated the US population requires more education on the subject of disease detection dogs. The variables that resulted in a moderate correlation in participants accepting implementation of COVID-19 scent detection dogs as a valid COVID-19 testing method included obtaining government approval and funding and implementing scent detection dogs as a more efficient and readily available worldwide testing method, particularly in developing countries that have restricted access to COVID-19 testing materials. The weakest

correlation results compared with other variables showed most participants remained reluctant to attend events with COVID-19 scent detection dogs present.

The strongest correlations observed were the different COVID-19 testing methods the participants experienced. A strong positive correlation was detected between the respondents who had been tested for COVID-19 and a PCR nasal swab test, which is consistent with the currently most prominent testing method available in the U.S. and the survey sample was representative of the U.S. population. In addition, a strong positive correlation resulted between experiencing testing via saliva sample and COVID-19 detection dogs. This suggested most participants had not experienced either one of these methods, further supporting the interpretation that RT-PCR testing via nasal swab is the most common COVID-19 testing method.

The PCA Scree Plot (Fig. 4) supported the PCA as an appropriate analysis method for the data set. The scree plot is an *a priori* analysis to ascertain if the majority of the data variability occurred within the first three principal components, allowing for further analysis to proceed. The PCA 1-2 biplot (Fig. 5) indicated age and education levels of the respondents likely had an important role in influencing the reliability of the different testing methods, such as how highly each participant rated the reliability of the results produced from a PCR test (Rel PCR), of an at-home testing kit (Rel home), and that of trained scent detection dogs (Rel dog). Additionally, it was found that age and education levels also played a key role in influencing the respondents' reported comfort levels with dogs. Based on the isolated position, age influenced the variables on the right side of the y-axis on the biplot (i.e., Ethn, Rel home, Rel dog, Rel PCR, Cmft dog, and Edu), but it can be inferred age did not influence any remaining variables tested in the survey. The variables exploring the COVID-19 test types experienced by the participants and which

testing methods resulted in COVID-19 positive results were all allied in the lower left quadrant of the bi-plot. These results showed these variables exhibited no influence on any other part of the survey. Consequently, it can be inferred the participants' experiences with COVID-19 and COVID-19 testing did not skew/influence any of the other variables tested.

The PCA 1-3 biplot (Fig. 6) revealed a more accurate depiction of the relationship between Cov test, all COVID-19 testing methods experienced, and which testing methods reported a positive COVID-19 result. Cov test was clustered with these variables. It is worth noting that it is often necessary to analyze data by looking at different components and dimensions for a more accurate understanding of a set of relationships. Likewise, the three-dimensional scatter plot (Fig. 7) further exemplified PCA does not serve as a flat two-dimensional analytical method.

UPGMA cluster analysis (Fig.8) results were congruent with CA and PCA. The clusters exhibited similar relationships between/among variables. According to this distance measure, most participants shared similar responses regarding the use of scent detection dogs for future pandemics and the viability of COVID-19 scent detection dogs for the recent Omicron BA.2 variant outbreak; the participants were clustered similarly regarding government approval and funding, and offering this testing method worldwide. Participants were clustered with the test types they experienced and the test types that resulted in a positive COVID-19 result. All of these relationships were also supported by the CA, demonstrating the data remained consistent across all analyses completed.

Each of the analytic methods (CA, PCA and UPGMA) shows promise for the future of COVID-19 detection dogs. With the evident grouping and significant correlations of these variables (i.e., Gov appvl, >viable, Emrg vrnt, Wrldwide, and Omi BA.2), it is safe to suggest the

majority of the survey participant population gave these questions notable consideration and with more outreach and education about disease detection dogs, particularly under pandemic and endemic disease conditions, would likely be in favor for implementing COVID-19 detection dogs worldwide.

Following the literature review and administered survey, one limitation was observed. The survey did not indicate how scent detection dogs detect COVID-19 from human individuals. These procedures cannot be assumed common knowledge, since education on this topic is certainly limited among the general population. Therefore, it is possible some of the participants did not have a clear understanding of the survey. Consequently, if given the knowledge of how scent detection dogs are trained to alert a positive detection of COVID-19, some of the participants might have responded differently.

VI.   Conclusions

Several recent studies (Jendrny et al. 2020; Gantz 2021; and Hag-Ali et al. 2021) were conducted to test scent detection dogs' accuracy to identify COVID-19. The results concluded trained scent detection dogs possessed the ability to perform with 90% to 99% accuracy. It was found dog detection showed a higher sensitivity than RT-PCR, but both tests demonstrated a high and similar specificity (99%) (Hag-Ali et al. 2021). The high specificity value of both testing methods indicated both techniques have great potential to accurately identify the truly negative COVID-19 individuals, yet dog detection tests are a quick and low-cost alternative to routine screening for COVID-19 with low transmission risk in comparison with PCR during sample collection (Hag-Ali et al. 2021). Scent detection dogs are also highly capable of "adapting to" (i.e. can be quickly trained to recognize new variants) the rapid evolutionary rates of SARS-CoV-2. Specifically, dogs are showing the ability to detect the Delta variant with up to

90% accuracy (Rodriquez 2021). Ultimately, trained scent detection dogs are showing great promise in identifying positive and negative cases of COVID-19 in human individuals. Scent detection dogs could be employed to slow the spread of COVID-19 more rapidly and efficiently given more research and training.

Future outreach to the general population is required to show the capabilities of these highly trained dogs, their success with COVID-19 detection, and potential in future pandemics. Public knowledge will facilitate a better understanding and a clearer decision for or against government approval and funding for the employment of COVID-19 scent detection dogs, for the present pandemic, endemic, and future pandemics.

Despite regulations lightening and lifting mask mandates worldwide, COVID-19 is far from over. Due to the evolutionary capabilities and nature of this disease, there is still an incredibly high potential for emerging variants. Although public outreach regarding COVID-19 detection dogs is essential, the survey results suggested many participants would be willing to implement detection dogs as a source of reliable COVID-19 testing. The evidence reported by research teams studying the efficacy of COVID-19 detection dogs and the responses gathered from the survey point to a bright future for detection dogs in our current and in future pandemics.

VII.   References Cited

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VIII.   Figure Legends

Fig. 1. RT-PCR based on the well-known TaqMan hydrolysis probes. (a) The probe is modified

with a fluorescent dye (reporter dye) at one end and one quencher dye on the other end. The

quencher blocks the fluorescent signal of the reporter dye due to proximity. The probe does not

fluoresce in its native condition. When the polymerase enzyme starts the amplification and

encounters the labeled probe, the probe gets hydrolyzed, releasing its components away from

each other, which results in a fluorescent signal. Each successful amplification creates

fluorescence that is proportional to the amount of the target gene in the sample. (b) Relative

positions of amplicon targets on SARS-CoV and Wuhan-CoV genome. S: Spike glycoprotein, E:

Envelope protein, M: Membrane protein, N: nucleocapsid; ORF: open reading frame; RdRp:

RNA-dependent RNA polymerase. Numbers below amplicon are genome positions according to

SARS-CoV.

Fig. 2. Canine's Anatomical Olfactory System.

Fig. 3. Correlation Analysis (CA). Each of the variables are along the y- and x-axes. The numeric

values are the *r-values* calculated between the two corresponding variables. Light grey shaded

boxes represent significant correlations with $P < 0.05$. Red shading represents negative

correlations and blue represents positive correlations.

Fig. 4. PCA Scree Plot.

Fig. 5. Principal Components Analysis 1-2 Biplot. Compares the first and second Principal Components

Fig. 6. Principal Components Analysis 1-3 Biplot. Compares the first and third Principal Components

Fig. 7. PCA 3-D Scatter Plot

Fig. 8. UPGMA Cluster Analysis

IX. Tables

Table 1. Demographic categories of the survey participants organized by ethnicity, sex, age, and education.

Demographic	Categorgy	Number of participants
Ethnicity	Asian	33
	Black	61
	Caucasian	322
	Mixed race	18
	Other	16
Sex	Female	222
	Male	220
	Non-binary,gender-fluid, or gender-queer	7
	Declined to answer	1
Age	18-28 years old	89
	29-39 years old	99
	40-50 years old	78
	51-61 years old	94
	62-72 years old	70
	73+ years old	20
Education	Less than a high school diploma	1
	High school degree or equivalent	56
	Some college education, but no degree	102
	Associate's degree	49
	Bachelor's degree	162
	Master's degree	62
	Professional degree	10
	Doctorate	8

Table 2. Calculated *r*-values and significance levels from survey data.

Variables	r-value	Correlation	Significance
Gov apprvl & Wrldwide	0.62	moderate positive	$P < 0.05$
Gov apprvl & Ctrl loc	0.45	weak positive	$P < 0.05$
Gov apprvl & Atten	0.27	weak positive	$P < 0.05$
Gov apprvl & Futr pan	0.41	weak positive	$P < 0.05$
Gov apprvl & Omi BA.2	0.37	weak positive	$P < 0.05$
Gov apprvl & Emrg vrnt	0.42	weak positive	$P < 0.05$
Gov apprvl & >viable	0.45	weak positive	$P < 0.05$
Gov apprvl & Rel dog	-0.35	weak negative	$P < 0.05$
Wrldwide & Ctrl loc	0.41	weak positive	$P < 0.05$
Wrldwide & Atten	0.22	weak positive	$P < 0.05$
Wrldwide & futr pan	0.42	weak positive	$P < 0.05$
Wrldwide & Omi BA.2	0.42	weak positive	$P < 0.05$
Wrldwide & Emrg vrnt	0.37	weak positive	$P < 0.05$
Wrldwide & >viable	0.36	weak positive	$P < 0.05$
Wrldwide Rel dog	-0.33	weak negative	$P < 0.05$
Ctrl loc & Atten	0.27	weak positive	$P < 0.05$
Ctrl loc & Futr pan	0.51	moderate positive	$P < 0.05$
Ctrl loc & Omi BA.2	0.48	weak positive	$P < 0.05$
Ctrl loc & Emrg vrnt	0.51	moderate positive	$P < 0.05$
Ctrl loc & >viable	0.40	weak positive	$P < 0.05$
Ctrl loc & Rel dog	-0.40	weak negative	$P < 0.05$
Atten & Futr pan	0.39	weak positive	$P < 0.05$
Atten & Omi BA.2	0.37	weak positive	$P < 0.05$
Atten & Emrg vrnt	0.28	weak positive	$P < 0.05$
Atten & >viable	0.17	weak positive	$P < 0.05$
Atten & Rel dog	-0.30	weak negative	$P < 0.05$
Futr pan & Omi BA.2	0.67	moderate positive	$P < 0.05$
Futr pan & Emrg vrnt	0.59	moderate positive	$P < 0.05$
Futr pan & >viable	0.39	weak positive	$P < 0.05$
Futr pan & Rel dog	-0.50	moderate negative	$P < 0.05$
Omi BA.2 & Emrg vrnt	0.61	moderate positive	$P < 0.05$
Omi BA.2 & >viable	0.32	weak positive	$P < 0.05$
Omi BA.2 & Rel dog	-0.50	moderate negative	$P < 0.05$
Emrg vrnt & >viable	0.51	moderate positive	$P < 0.05$

Emrg vrnt & Rel dog	-0.55	moderate negative	$P < 0.05$
>viable & Rel dog	-0.33	weak negative	$P < 0.05$

Table 3. Survey variable abbreviation legend

Variable Abbreviation	Survey Question Asked
Sex	Which of the following terms best describes your gender identity?
Ethn	Which of the following terms best describes your ethnicity?
Age	Indicate your age.
Edu	What is the highest degree or level of school you have completed?
Cmft dog	On the scale below, rate your comfort level with dogs.
Rel PCR	On the scale below, how reliable would you rank a COVID-19 diagnosis from a Polymerase Chain Reaction (PCR) test via nasal swab?
Rel home	On the scale below, how reliable would you rank a COVID-19 diagnosis from an at-home testing kit?
Rel dog	On the scale below, how reliable would you rank a COVID-19 diagnosis from a trained scent detection dog 'sniffing out' the virus?
>viable	At this point in the pandemic, would you feel the detection of COVID-19 by a trained scent detection dog would be more viable and readily available approach?
Cov Test	Have you ever been tested for COVID-19?
Nas test	If tested for COVID-19, did you experience testing via nasal swab?
Sal test	If tested for COVID-19, did you experience testing via saliva sample?
Dog test	If tested for COVID-19, did you experience testing via scent dog detection?
Pos Cov	Have you received a positive result from a COVID-19 test?
Nas Pos	If you received a positive result from a COVID-19 test, was collection via the nasal swab method that resulted in a positive result?
Sal Pos	If you received a positive result from a COVID-19 test, was collection via saliva sample the method used that resulted in a positive result?
Dog Pos	If you received a positive result from a COVID-19 test, was detection via scent detection dog that resulted in a positive result?
Emrg vrnt	In terms of potential for emerging COVID-19 variants (such the Omicron variant), do you feel scent dog detection is a more viable testing approach?
Omi BA.2	Some of the most recent research has led scientists to a second strand of the Omicron variant that is even more transmissible than the first and has been traced to evade the COVID-19 vaccines. Knowing that rapid detection of positive cases is critical to stop the spread, do you think the instantaneous detection from a scent detection dog would be a valid solution to such a problem?
Futr pan	In terms of future pandemics, if scent detection dogs and their handlers were employed at public locations where mass gatherings are common, such as

	sporting events, airports, concert halls, etc., do you feel this would be a reliable testing method?
Atten	If scent detection dogs and their handlers were present at the previously described public areas, would you still attend such events?
Ctrl loc	If there was a centralized location in your city with a detection dog and its handler with the sole purpose of identifying COVID-19 positive patients, such as at the Hospital of Sarasota in Sarasota, Florida, would you seek out this immediate testing method over others, such as at-home testing kits or the RT-PCR tests?
Gov appvl	Research shows scent detection dogs are as effective as PCR in detecting COVID-19, but hurdles remain in widespread implementation. Do you support government approval, with standards established, for official COVID-19 scent dog detection and subsequent funding to initiate a program for widespread use in the US?
Wrldwide	Should scent detection dogs be used worldwide, particularly targeting underdeveloped countries without adequate access to laboratory materials required for Polymerase Chain Reaction (PCR) testing methods and test kits, i.e. individuals are tested once symptomatic, leading to the emergence of variants?

X. Figures

a) The principle of TaqMan probe based RT-PCR
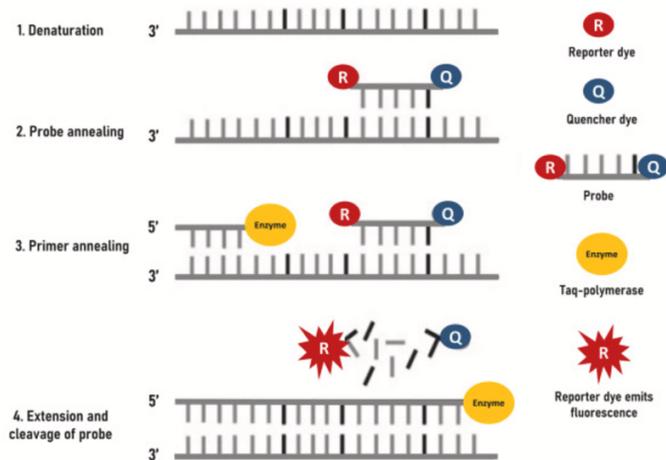
b) Relative positions of amplicon targets for SARS-CoV-2 RT-PCR

Fig. 1. RT-PCR based on the well-known TaqMan hydrolysis probes. (a) The probe is modified

with a fluorescent dye (reporter dye) at one end and one quencher dye on the other end. The

quencher blocks the fluorescent signal of the reporter dye due to proximity. The probe does not

fluoresce in its native condition. When the polymerase enzyme starts the amplification and

encounters the labeled probe, the probe gets hydrolyzed, releasing its components away from

each other, which results in a fluorescent signal. Each successful amplification creates

fluorescence that is proportional to the amount of the target gene in the sample. (b) Relative

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Envelope protein, M: Membrane protein, N: nucleocapsid; ORF: open reading frame; RdRp:

RNA-dependent RNA polymerase. Numbers below amplicon are genome positions according to

SARS-CoV.

Figure 2. Canine's Anatomical Olfactory System.

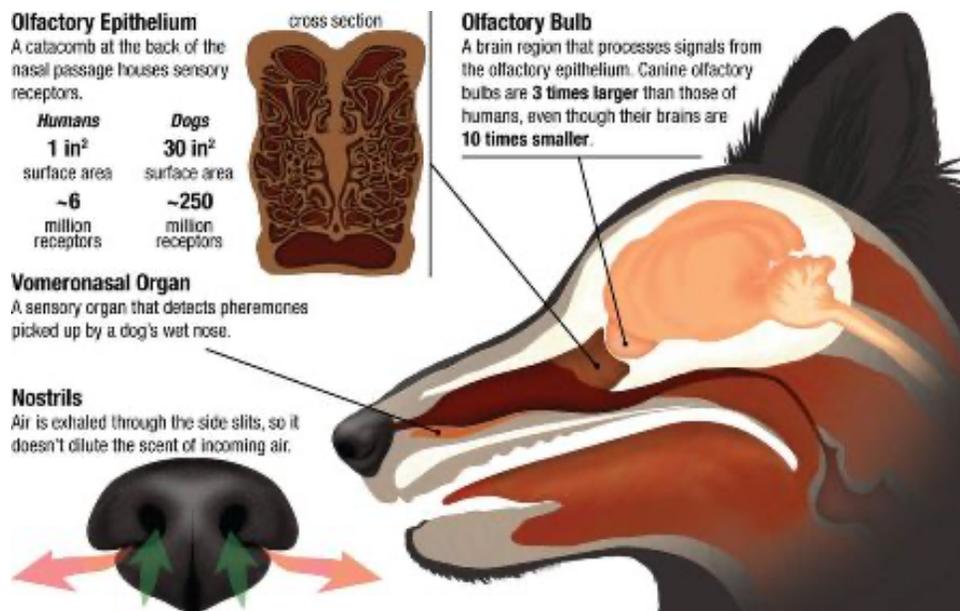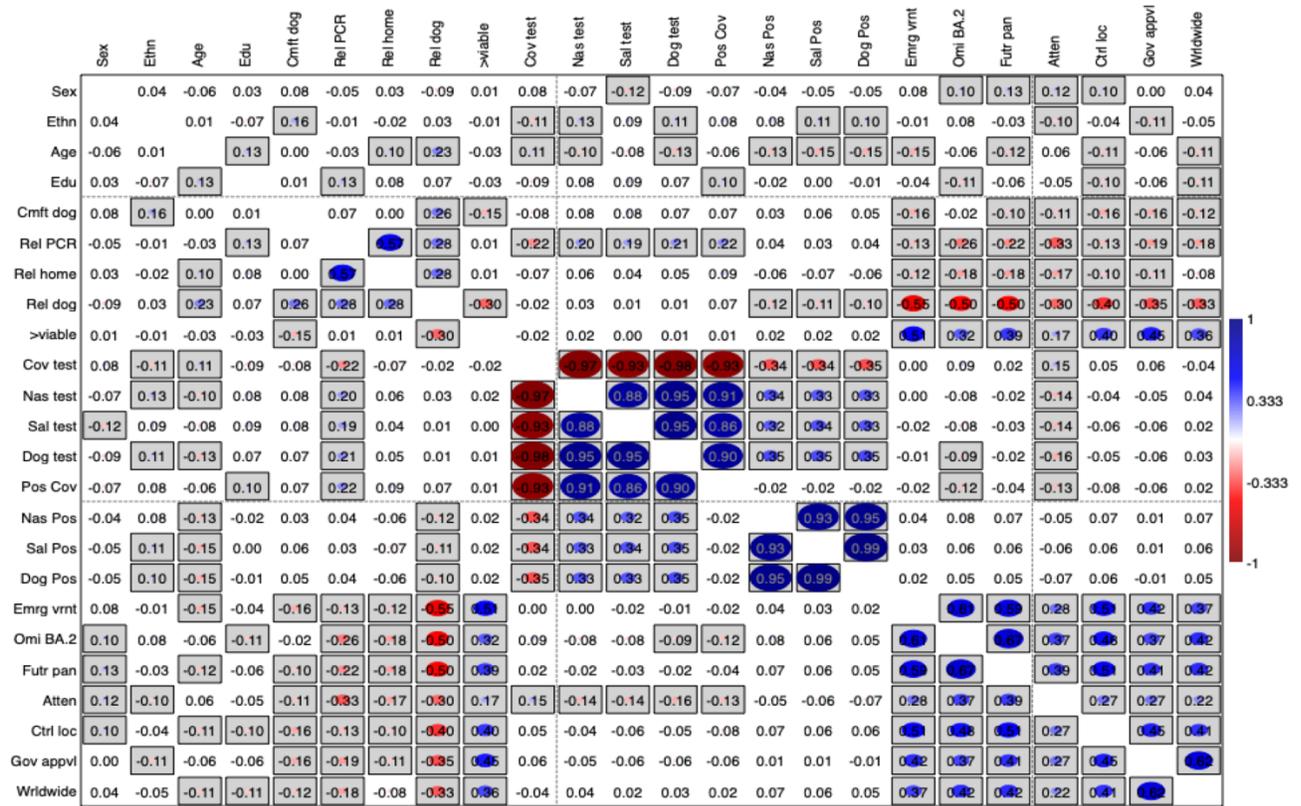|	Sex	Ethn	Age	Edu	Cmft dog	Rel PCR	Rel home	Rel dog	>viable	Cov test	Nas test	Sal test	Dog test	Pos Cov	Nas Pos	Sal Pos	Dog Pos	Emrg vrnt	Omi BA.2	Futr pan	Atten	Ctrl loc	Gov appvl	Wrldwide
Sex		0.04	-0.06	0.03	0.08	-0.05	0.03	-0.09	0.01	0.08	-0.07	-0.12	-0.09	-0.07	-0.04	-0.05	-0.05	0.08	0.10	0.13	0.12	0.10	0.00	0.04
Ethn	0.04		0.01	-0.07	0.16	-0.01	-0.02	0.03	-0.01	-0.11	0.13	0.09	0.11	0.08	0.08	0.11	0.10	-0.01	0.08	-0.03	-0.10	-0.04	-0.11	-0.05
Age	-0.06	0.01		0.13	0.00	-0.03	0.10	0.23	-0.03	0.11	-0.10	-0.08	-0.13	-0.06	-0.13	-0.15	-0.15	-0.15	-0.06	-0.12	0.06	-0.11	-0.06	-0.11
Edu	0.03	-0.07	0.13		0.01	0.13	0.08	0.07	-0.03	-0.09	0.08	0.09	0.07	0.10	-0.02	0.00	-0.01	-0.04	-0.11	-0.06	-0.05	-0.10	-0.06	-0.11
Cmft dog	0.08	0.16	0.00	0.01		0.07	0.00	0.26	-0.15	-0.08	0.08	0.08	0.07	0.07	0.03	0.06	0.05	-0.16	-0.02	-0.10	-0.11	-0.16	-0.16	-0.12
Rel PCR	-0.05	-0.01	-0.03	0.13	0.07		0.57	0.28	0.01	-0.22	0.20	0.19	0.21	0.22	0.04	0.03	0.04	-0.13	-0.26	-0.22	-0.33	-0.13	-0.19	-0.18
Rel home	0.03	-0.02	0.10	0.08	0.00	0.57		0.28	0.01	-0.07	0.06	0.04	0.05	0.09	-0.06	-0.07	-0.06	-0.12	-0.18	-0.18	-0.17	-0.10	-0.11	-0.08
Rel dog	-0.09	0.03	0.23	0.07	0.26	0.28	0.28		-0.30	-0.02	0.03	0.01	0.01	0.07	-0.12	-0.11	-0.10	-0.55	-0.50	-0.50	-0.30	-0.40	-0.35	-0.33
>viable	0.01	-0.01	-0.03	-0.03	-0.15	0.01	0.01	-0.30		-0.02	0.02	0.00	0.01	0.01	0.02	0.02	0.02	0.51	0.32	0.39	0.17	0.40	0.45	0.36
Cov test	0.08	-0.11	0.11	-0.09	-0.08	-0.22	-0.07	-0.02	-0.02		-0.97	-0.93	-0.98	-0.93	-0.34	-0.34	-0.35	0.00	0.09	0.02	0.15	0.05	0.06	-0.04
Nas test	-0.07	0.13	-0.10	0.08	0.08	0.20	0.06	0.03	0.02	-0.97		0.88	0.95	0.91	0.34	0.33	0.33	0.00	-0.08	-0.02	-0.14	-0.04	-0.05	0.04
Sal test	-0.12	0.09	-0.08	0.09	0.08	0.19	0.04	0.01	0.00	-0.93	0.88		0.95	0.86	0.32	0.34	0.33	-0.02	-0.08	-0.03	-0.14	-0.06	-0.06	0.02
Dog test	-0.09	0.11	-0.13	0.07	0.07	0.21	0.05	0.01	0.01	-0.98	0.95	0.95		0.90	0.35	0.35	0.35	-0.01	-0.09	-0.02	-0.16	-0.05	-0.06	0.03
Pos Cov	-0.07	0.08	-0.06	0.10	0.07	0.22	0.09	0.07	0.01	-0.93	0.91	0.86	0.90		-0.02	-0.02	-0.02	-0.02	-0.12	-0.04	-0.13	-0.08	-0.06	0.02
Nas Pos	-0.04	0.08	-0.13	-0.02	0.03	0.04	-0.06	-0.12	0.02	-0.34	0.34	0.32	0.35	-0.02		0.93	0.95	0.04	0.08	0.07	-0.05	0.07	0.01	0.07
Sal Pos	-0.05	0.11	-0.15	0.00	0.06	0.03	-0.07	-0.11	0.02	-0.34	0.33	0.34	0.35	-0.02	0.93		0.99	0.03	0.06	0.06	-0.06	0.06	0.01	0.06
Dog Pos	-0.05	0.10	-0.15	-0.01	0.05	0.04	-0.06	-0.10	0.02	-0.35	0.33	0.33	0.35	-0.02	0.95	0.99		0.02	0.05	0.05	-0.07	0.06	-0.01	0.05
Emrg vrnt	0.08	-0.01	-0.15	-0.04	-0.16	-0.13	-0.12	-0.55	0.51	0.00	0.00	-0.02	-0.01	-0.02	0.04	0.03	0.02		0.61	0.61	0.28	0.61	0.42	0.37
Omi BA.2	0.10	0.08	-0.06	-0.11	-0.02	-0.26	-0.18	-0.50	0.32	0.09	-0.08	-0.08	-0.09	-0.12	0.08	0.06	0.05	0.61		0.61	0.37	0.61	0.37	0.42
Futr pan	0.13	-0.03	-0.12	-0.06	-0.10	-0.22	-0.18	-0.50	0.39	0.02	-0.02	-0.03	-0.02	-0.04	0.07	0.06	0.05	0.61	0.61		0.39	0.61	0.41	0.42
Atten	0.12	-0.10	0.06	-0.05	-0.11	-0.33	-0.17	-0.30	0.17	0.15	-0.14	-0.14	-0.16	-0.13	-0.05	-0.06	-0.07	0.28	0.37	0.39		0.27	0.27	0.22
Ctrl loc	0.10	-0.04	-0.11	-0.10	-0.16	-0.13	-0.10	-0.40	0.40	0.05	-0.04	-0.06	-0.05	-0.08	0.07	0.06	0.06	0.61	0.61	0.61	0.27		0.45	0.41
Gov appvl	0.00	-0.11	-0.06	-0.06	-0.16	-0.19	-0.11	-0.35	0.45	0.06	-0.05	-0.06	-0.06	-0.06	0.01	0.01	-0.01	0.42	0.37	0.41	0.27	0.45		0.61
Wrldwide	0.04	-0.05	-0.11	-0.11	-0.12	-0.18	-0.08	-0.33	0.36	-0.04	0.04	0.02	0.03	0.02	0.07	0.06	0.05	0.37	0.42	0.42	0.22	0.41	0.61	

Fig. 3. Correlation Analysis (CA). Each of the variables are along the y- and x-axes. The numeric values are the *r-values* calculated between the two corresponding variables. Light grey shaded boxes represent significant correlations with $P < 0.05$. Red shading represents negative correlations and blue represents positive correlations.
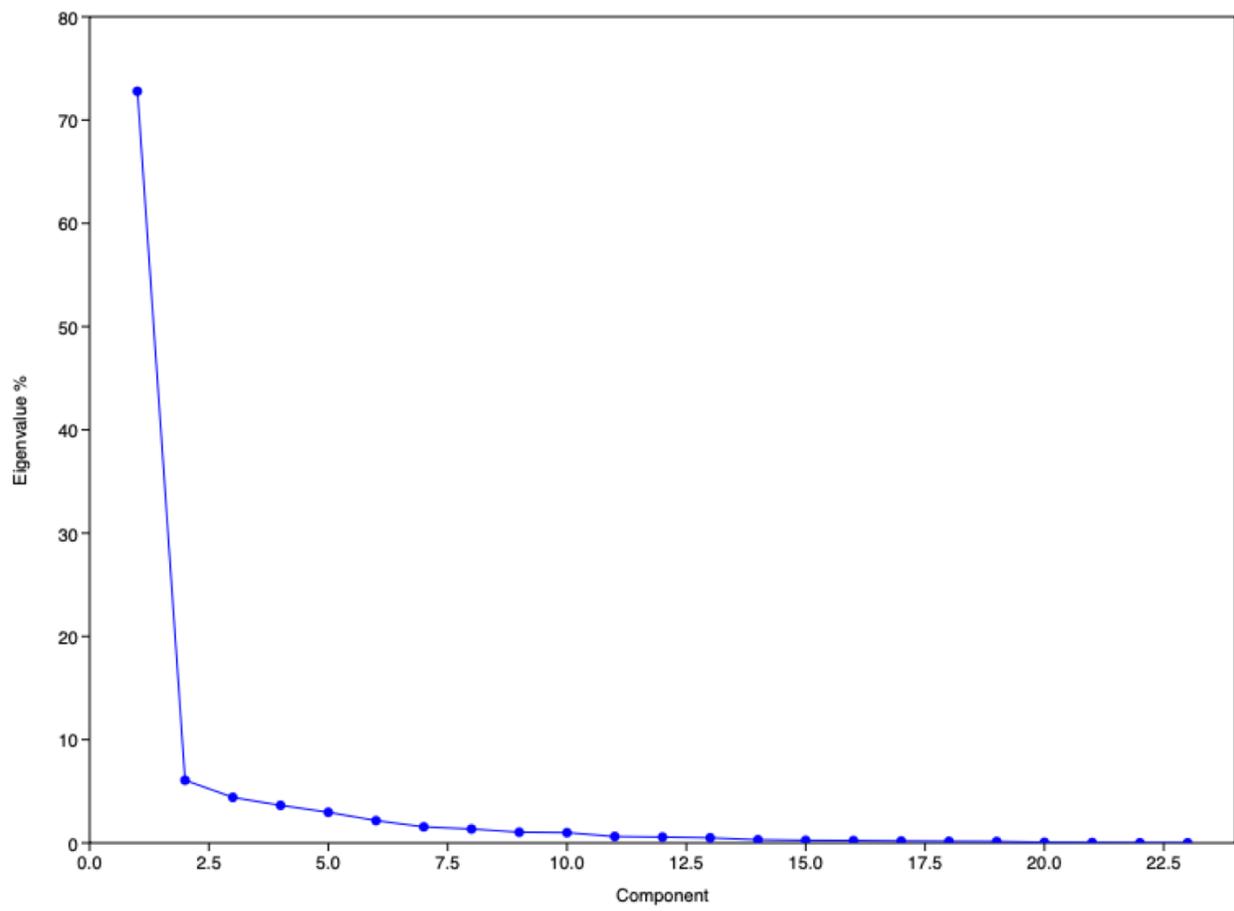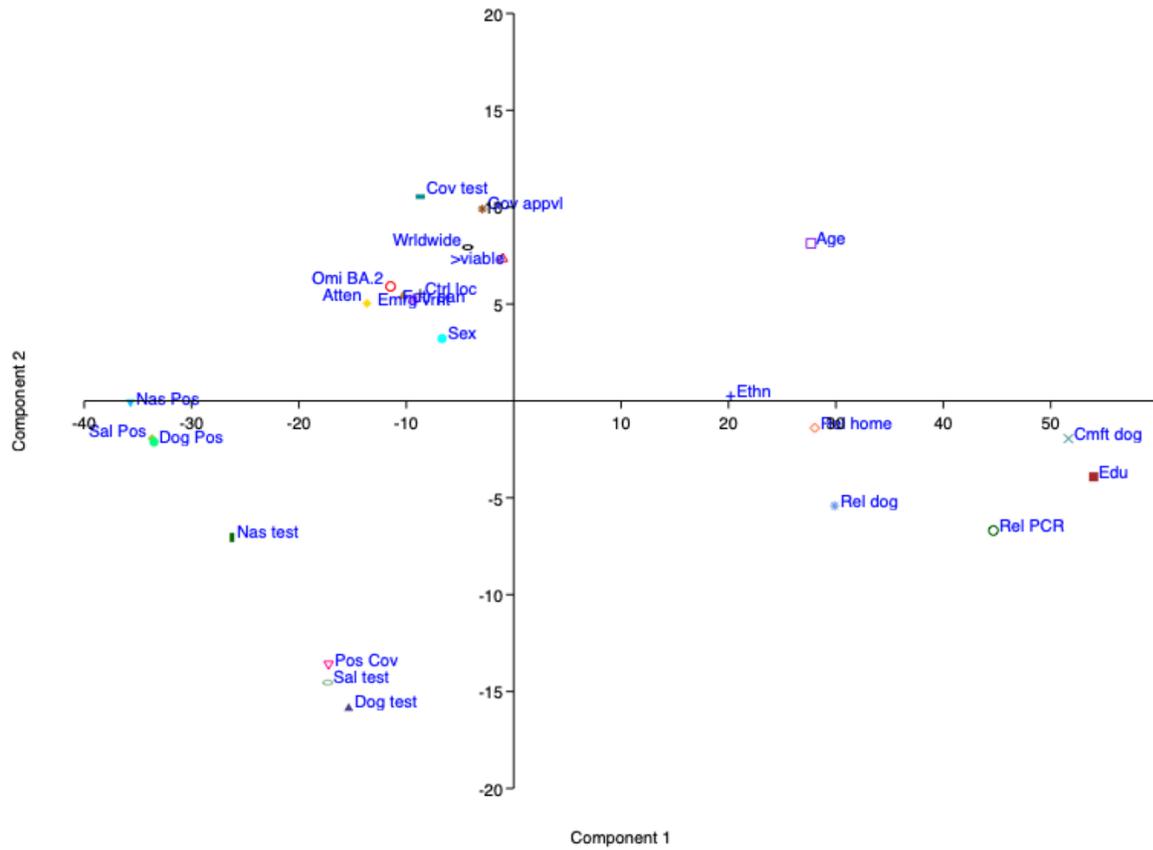
Figure 4. PCA Scree Plot.

Fig. 5. Principal Components Analysis 1-2 Biplot. Compares the first and second Principal
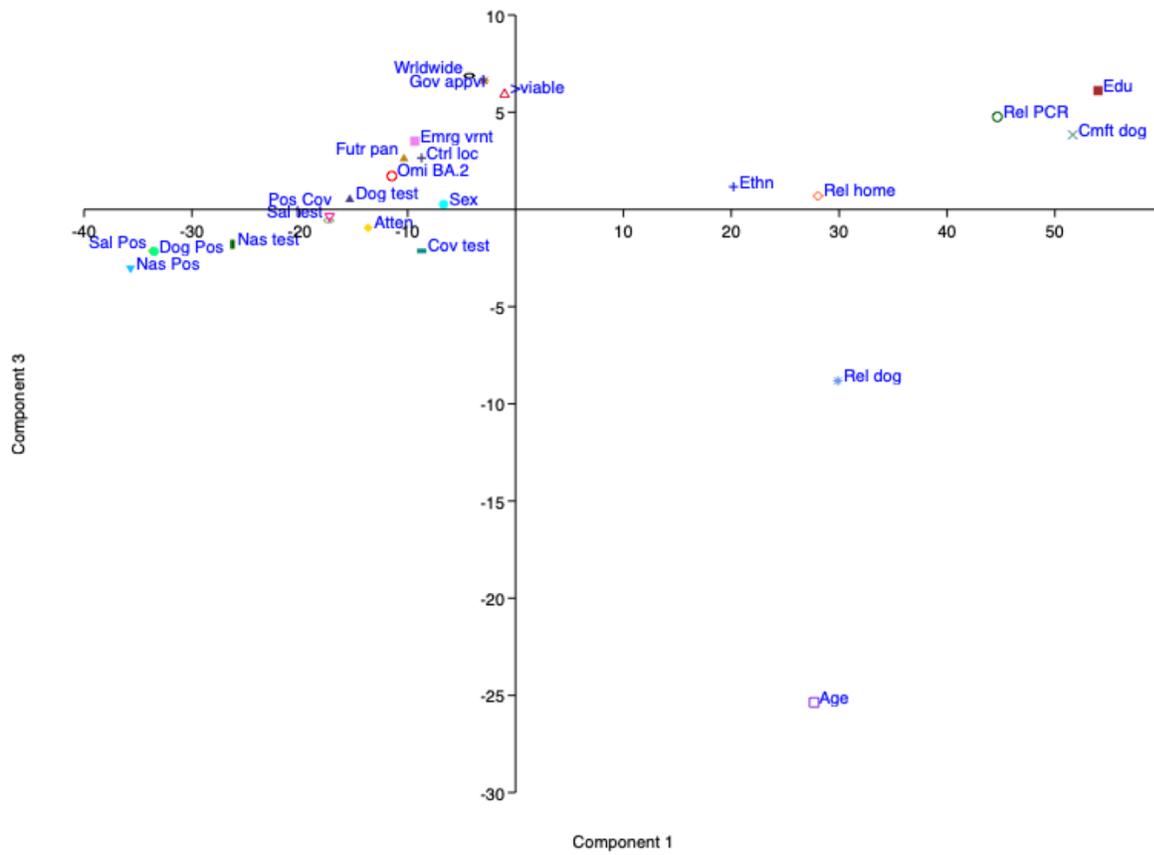
Components

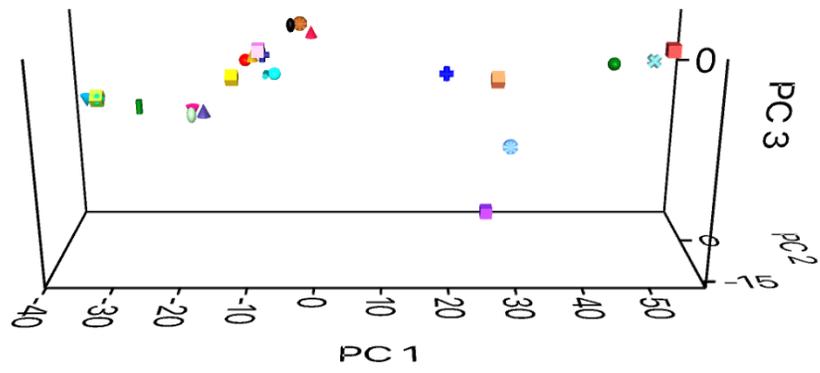Fig. 6. Principal Components Analysis 1-3 Biplot. Compares the first and third Principal Components
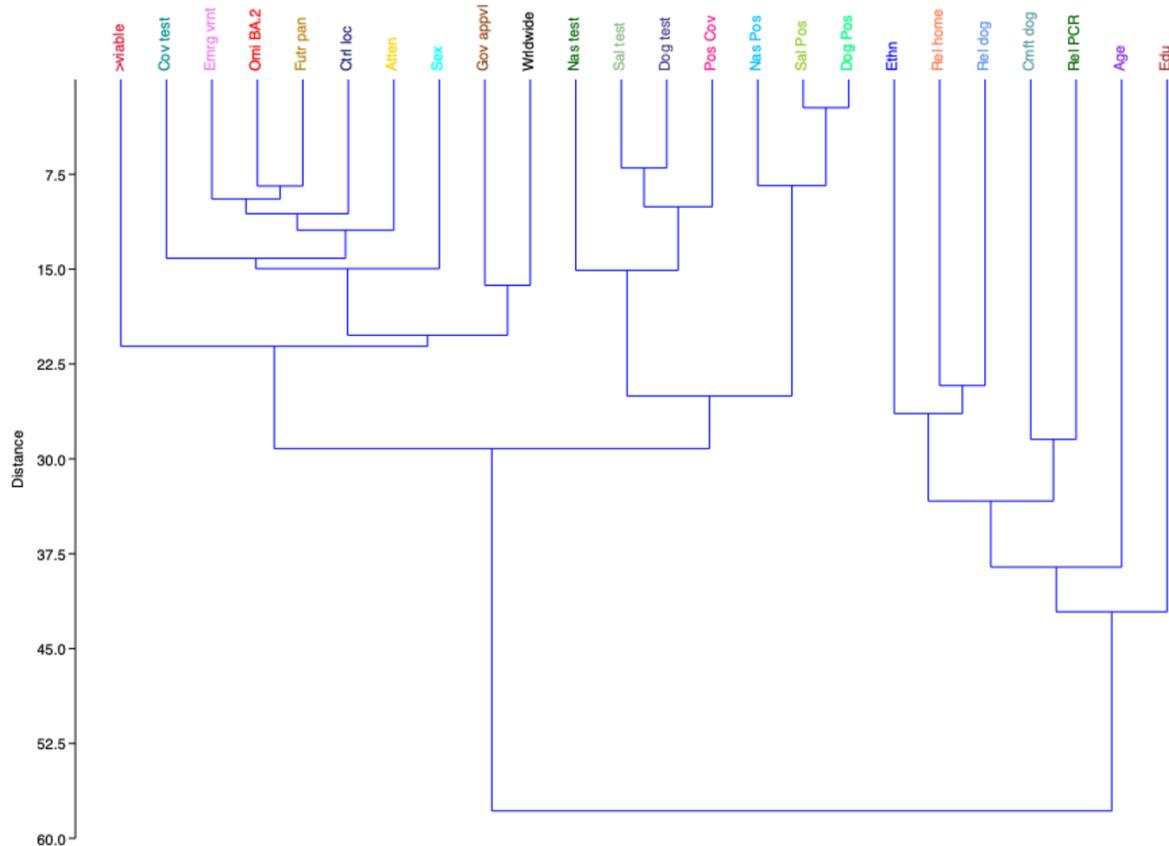
Fig. 7. PCA 3-D Scatter Plot

Figure 8. UPGMA Cluster Analysis

XI. Appendix

(1) Survey

1. Which of the following terms **best** describes your current gender identity? We understand that there are many different ways you may identify, please pick the one that **best** describes you.
   a. Female
   b. Male
   c. Nonbinary, genderfluid, or genderqueer
   d. I am not sure or questioning
   e. Decline to answer

2. Indicate your age: _____
   a. 18-28 years old
   b. 29-39 years old
   c. 40-50 years old
   d. 51-61 years old
   e. 62-72 years old

      f.   73 years or older

3. What is the highest degree or level of school you have completed? If currently enrolled, indicate the highest degree received: _____
   a. Less than a high school diploma
   b. High school degree or equivalent (e.g. GED)
   c. Some college, no degree
   d. Associate degree (e.g. AA, AS)
   e. Bachelor's degree (e.g. BA, BS)
   f. Master's degree (e.g. MA, MS, MEd)
   g. Professional degree (e.g. MD, DDS, DVM)
   h. Doctorate (e.g. PhD, EdD)

4. On the scale below, rate your comfort level with dogs.

1	2	3	4	5
Very uncomfortable	Uncomfortable	Average	Comfortable	Very comfortable

5. On the scale below, how reliable would you rank a COVID-19 diagnosis from a Polymerase Chain Reaction (PCR) test via a nasal swab?

1	2	3	4	5
Very unreliable	Unreliable	Average	Reliable	Very reliable

6. On the scale below, how reliable would you rank a detection of COVID-19 diagnosis from an at-home testing kit?

1	2	3	4	5
Very unreliable	Unreliable	Average	Reliable	Very reliable

7. On the scale below, how reliable would you rank a COVID-19 diagnosis from a trained scent detection dog 'sniffing out' the virus?

1	2	3	4	5
Very unreliable	Unreliable	Average	Reliable	Very reliable

8. Several recent studies (Jendrny et al. 2020; Gantz 2021; & Hag-Ali et al. 2021) were conducted to test scent detection dogs' accuracy to identify COVID-19. The results concluded trained scent dogs performed with 90% to 99% accuracy. At this point in the pandemic, would you feel the detection for COVID-19 by trained scent detection dogs is a more viable and readily available approach?
_____ Yes   _____ No   _____ I'm not sure

9. Have you ever been tested for COVID-19? If no, skip to question 13.

_____ Yes   _____ No

10. If tested for COVID-19, did you experience testing via nasal swab?
_____ Yes   _____ No   _____ NA

If tested for COVID-19, did you experience testing via saliva sample?
_____ Yes   _____ No   _____ NA

If tested for COVID-19, did you experience testing via scent dog detection?
_____ Yes   _____ No   _____ NA

11. Have you received a positive result from a COVID-19 test? If no, skip to question 13.
_____ Yes   _____ No

12. If you received a positive result from a COVID-19 test, was collection via the nasal swab method that resulted in a positive result?
_____ Yes   _____ No   _____ NA

If you received a positive result from a COVID-19 test, was collection via saliva sample the method used that resulted in a positive result?
_____ Yes   _____ No   _____ NA

If you received a positive result from a COVID-19 test, was detection via scent detection dog that resulted in a positive result?
_____ Yes   _____ No   _____ NA

13. In terms of potential for emerging COVID-19 variants (such the Omicron variant), do you feel scent dog detection is a more viable testing approach?
_____ Yes   _____ No

14. Some of the most recent research has led scientists to a second strand of the Omicron variant that is even more transmissible than the first and has been traced to evade the COVID-19 vaccines. Knowing that rapid detection of positive cases is critical to stop the spread, do you think the instantaneous detection from a scent detection dog would be a valid solution to such a problem?
_____ Yes   _____ No

15. In terms of future pandemics, if scent detection dogs and their handlers were employed at public locations where mass gatherings are common, such as sporting events, airports, concert halls, etc., do you feel this would be a reliable testing method?

____ Yes   ____ No

16. If scent detection dogs and their handlers were present at the previously described public areas, would you still attend such events?
    ____ Yes   ____ No

17. If there was a centralized location in your city with a detection dog and its handler with the sole purpose of identifying COVID-19 positive patients, such as at the Hospital of Sarasota in Sarasota, Florida, would you seek out this immediate testing method over others, such as at-home testing kits or the RT-PCR tests?
    ____ Yes   ____ No

18. Research shows scent detection dogs are as effective as PCR in detecting COVID-19, but hurdles remain in widespread implementation. Do you support government approval, with standards established, for official COVID-19 scent dog detection and subsequent funding to initiate a program for widespread use in the US?
    ____ Yes   ____ No   ____ I'm not sure

19. Should scent detection dogs be used worldwide, particularly targeting underdeveloped countries without adequate access to laboratory materials required for Polymerase Chain Reaction (PCR) testing methods and test kits, i.e. individuals are tested once symptomatic, leading to the emergence of variants?
    ____ Yes   ____ No   ____ I'm not sure

(2) Dog Detection Training using the Detection Dog Training System (Jendrny et al. 2020).