UNIVERSITY OF VETERINARY MEDICINE BUDAPEST

Institute for Animal breeding, Nutrition and Laboratory Animal Science Department for Veterinary Genetics and Animal Breeding

Medical Detection Dog

DIPLOMA WORK

Written by

Avigail Sala Simon

Supervisor: Prof. Dr. László Zöldág

Budapest, 2023

Table of contact

2. list of abbreviations

Biological detective dog - BDD

Detective dog – DD

Dichlorodiphenyltrichloroethane – DDT

Main olfactory epithelium - MOE

Obstructive sleep apnea – OSA

Olfactory bulb - OB

Olfactory receptor – OR

Olfactory receptor cells – ORC

The vomeronasal organ - VNO

Volatile organic compound - VOC

3. Introduction

Dogs were the first animal to be domesticated. Over the years it has been revealed, through genetic and phenotypic research, that dogs originated from an extinct gray wolf population and were domesticated in the Old World.
Due to inconsistencies in studies, ideas and methodologies there is still an ongoing debate regarding the date and geographic origin of the domestication of dogs. Fortunately, growing evidence can shed new light on the origin and evolutionary course of the dog. The latest research evidence predates the beginning of domestication to be approximately 33,000 years ago in East Asia, where the oldest known dog skeletons were found.
Research on ancient DNA has suggested that dogs may have been domesticated independently in Eastern and Western Eurasia from distinct wolf populations approximately 15,000 years ago. American dogs are likely to have originated from East Asia and have spread to the Americas alongside people. However, they almost completely disappeared after the arrival of Europeans, leaving the closest detectable genetic legacy around 8,000 years ago.

Over the past few centuries, the breeding processes among domesticated dogs have accelerated, resulting in the development of nearly 400 breeds that fulfill specialized functions in human society. Those breeds exhibit clear morphological and behavioral differences, depending on the need they had to fulfill, whether it be to help retrieve game in hunting, to search for food in small areas, or simply to be companions, dogs have gone through multiple physical or anatomical changes.

Since the late 19th century, it has been documented that dogs were used in the police force in Belgium and this practice eventually spread to other European countries. As the research progressed, it was discovered that dogs had an exceptional sense of smell which could be utilized in various fields. Over the past three decades, their olfactory abilities have been highly beneficial in the field of medical research.

3.1 The Anatomy, Histology, and Physiology of canine olfactory organ

Most mammals, dogs included, have an olfactory system that is composed from the main olfactory epithelium (MOE), the vomeronasal organ (VNO), and the olfactory bulb (OB).
The nasal cavity consists of two chambers vascularized by the sphenopalatine artery. The chamber is separated and divided in two by the nasal septum. Each chamber contains three turbinates: nasoturbinate, maxilloturbinate, and ethmoturbinate that increase the mucosal surface area. The caudo-dorsal region of the nasal cavity, lined by the MOE, is the usually pigmented part of the mucosa, while the VNO is located just above the roof of the mouth, where the vomer bone is. The VNO lies between the two main cavities of the head, the nasal cavity and the oral cavity. It is a tubular elongated organ which is separated by the nasal septum. It is connected to the mouth through the nasopalatine duct, which opens on the palate and behind the upper incisors.

The olfactory epithelium is composed of ORC, which are bipolar neurons. These cells project out and interact with the odor particles together with the sustentacular and basal cells.
Pluripotent basal cells can be differentiated into sustentacular cells or ORC, which have a short lifespan of a few weeks, estimated between 30-60 days. However, the ORC are constantly regenerating from the pluripotent basal cells. The sustentacular cells are the structural support that surrounds the ORC. They take part in the phagocytosis of dead neurons, odorant and xenobiotic particle transformation.
The olfactory gland, also named the Bowman's gland, is present in the olfactory epithelium mucosa, it protects the neuroepithelium from toxic and dangerous particles that exist in the air and prevent them from entering the nasal cavity, by forming a secretion that causes the odor particles to dissolve. The gland also maintains the normal humidity environment in the nasal cavity. The humidity is the key factor that traps the odorant particles and maintains normal olfactory perception.

Cilia with odor receptors cover the ORC. Dogs possess hundreds of cilia per ORC, compared to an estimated 25 cilia in humans. This fact allows dogs to analyze substantially smaller concentrations of odorant particles. Their nasal cavity contains more than 220 million ORs, enabling them to detect an enormous number of odorants. There is only one type of OR per ORC, a different unique combination of triggered receptors that seems to react to individual odors. The intensity of odor is proportional to the number of activated ORCs as well as the time of exposure to the smell. ORCs also have receptors for hormones and neurotransmitters.

In the membrane of the ORC cilia there are extracellular proteins that bind odorant and intracellular proteins that are linked to G-protein. When an odorant connects with the extracellular portion of the receptor, the structure of the G-protein breaks away and triggers adenylyl cyclase, which transforms ATP into cAMP. The signal from the odorant is amplified by cAMP, which activates numerous sodium-gated channels. The sodium channels open in two steps, causing depolarization, and the resulting action potential travels through the olfactory bulb. Each OR is activated by a distinct combination of odorants. This mechanism is the basis for a detection dog's ability to recognize odors accurately.

When an odor is detected, the olfactory nerve, also known as the first cranial nerve, generates an impulse that is transmitted to the cribriform plate which is located on the ethmoid bone.
These olfactory pathways continue with the next organ, the olfactory bulb, which is found under the frontal lobes. The olfactory bulb (OB) is composed of bundles of nerve fibers that come together to form the glomeruli. In this organ, the incoming receptor cell axons interact with the mitral neuron dendrites through a synaptic connection. Then the axon transmits to the other part of the brain the impulse

The olfactory bulb plays a crucial role in processing and filtering olfactory information, helping to distinguish between odors and enhancing sensitivity to odor detection while filtering out background odors. Unlike other senses, where the sensory track intersects, olfactory pathways lead from the detection area in the nasal cavity to the perception area in the brain on the same side in the brain. The right nostril sends signals to the right brain hemisphere, while the left nostril sends impulses to the left hemisphere. From the OB, olfactory signals travel to the olfactory cortex, which includes the anterior olfactory cortex, piriform cortex, periamygdaloid cortex, and the entorhinal cortex. The first three transmit the olfactory signal to the frontal cortex and thalamus, while the entorhinal cortex sends impulses to the hippocampal formation, which takes part in memory recognition of odors. The thalamus is believed to be responsible for odor thresholding.

The main olfactory epithelium and vomeronasal organs are separated in the collection of smell signals, and specific signals that they react to. The pathways leading to the brain also separate signals perceived by the MOE and VNO. These structures can be used to detect different kinds of stimuli. The VNO is the primary structure responsible for pheromone recognition, but can also recognize other low VOC.

Detecting smell begins with inhaling air that contains VOCs with varying compositions. VOCs can have different magnitudes, volatilities, and concentrations. The concentration of odor in the air is linked to the concentration of the odor source, volatility, odor-releasing surface area of the source, volume flow rate, ambient air movements, and diffusion velocity within the source. Biogenic VOCs, such as isoprene and monoterpenes, alkanes, alkenes, carbonyls, alcohols, esters, ethers, and acids, have strong odors and are produced and emitted by animals, plants, and micro-organisms. Over 1000 VOCs are liberated from various tissues and body fluids, with the most common being skin emanations, urine, blood, saliva, and feces, which differ in their VOC composition. Other VOCs may come from foods, drinks, medications, personal hygiene products, and pollutants that are ingested, inhaled, or absorbed through the skin. Organisms emit an extensive repertoire of VOCs that vary with age, diet, gender, genetics, and physiological or pathological status. A combination of endogenous and exogenous VOCs is present and should be labeled as disease biomarkers. Animals, humans, insects, and even plants can detect VOCs that are released from an organism. Various possibilities of measuring specific VOCs exist, such as gas chromatography mass-spectrometric techniques (GC-MS) for identification and characterization. Recent research has demonstrated that volatile can be used to detect disease, pathogens, and many other unique aspects of an organism.

When air is inhaled, there are two main pathways routes; in every sniff 12%-13% of the air goes to the upper flow path that leads to the olfactory area, where odor molecules residue accumulates. The lower pathway contains the remaining air entering the lungs through the pharynx, this route is also used for expiration. The turbulence in the air flow is a result of anatomical and physiological factors. These factors also influence the humidity, temperature, and path of the inhaled air, and guide the part of the air through the olfactory epithelium.

During inhalation in each nostril, odor particle samples are obtained, analyzed, compared, and measured, allowing the dog to understand the source. In dogs, as well as in humans, the smelling processes cause a lateralization that can be observed in different regions of each hemisphere of the brain. The right hemisphere is stimulated when a new odor has been inhaled, therefore strongly controlling the sympathetic nervous system and hypothalamic–pituitary–adrenal axis. Meanwhile, the left hemisphere regulates native response for stimulation. The sniffing starts with the right nostril, which is the dominant one, then, if the odor is recognized as a delightful or familiar smell like a snack or the dog's favorite food the smell route changes and the left nostril gets involved with the sniffing. If the odor is recognized as unfamiliar, frightening, or stimulating such as the smell of Adrenaline only the right nostril is used.

Compared to other species, the VNO of dogs lacks the VR2-based vomeronasal subsystem. This has been confirmed by the observation that the canine system does not express Gαo and expresses Gαi2 throughout the accessory olfactory bulb as well as in the vomeronasal sensory epithelium (VNsE). Additionally, the lack of functional VR2 genes in the canine genome further supports this observation. Unlike many other mammals, dogs do not have additional organs responsible for chemoreception such as Masera's septal olfactory organ in cats or the Grüneberg ganglion that is a chemo and thermos sense organ in some mammalian species such as rodents. Although the latter has been mistakenly shown to be a part of the canine chemoreception system, it has not yet been identified in dogs. While the Grüneberg ganglion might possibly be present in early embryonic stages, it seems to regress during prenatal development.
In humans, the detection of volatile chemicals is mediated by both the olfactory and trigeminal systems. Odors can only be detected when the olfactory stimulants simultaneously excite the trigeminal somatosensory system. However, in dogs, odor detection is implemented only through the olfactory neuroepithelium. While some

odors can stimulate the nasal mucosa trigeminal nerve endings in dogs, detect feelings such as warmth or coolness, odor detection is primarily through the olfactory system.

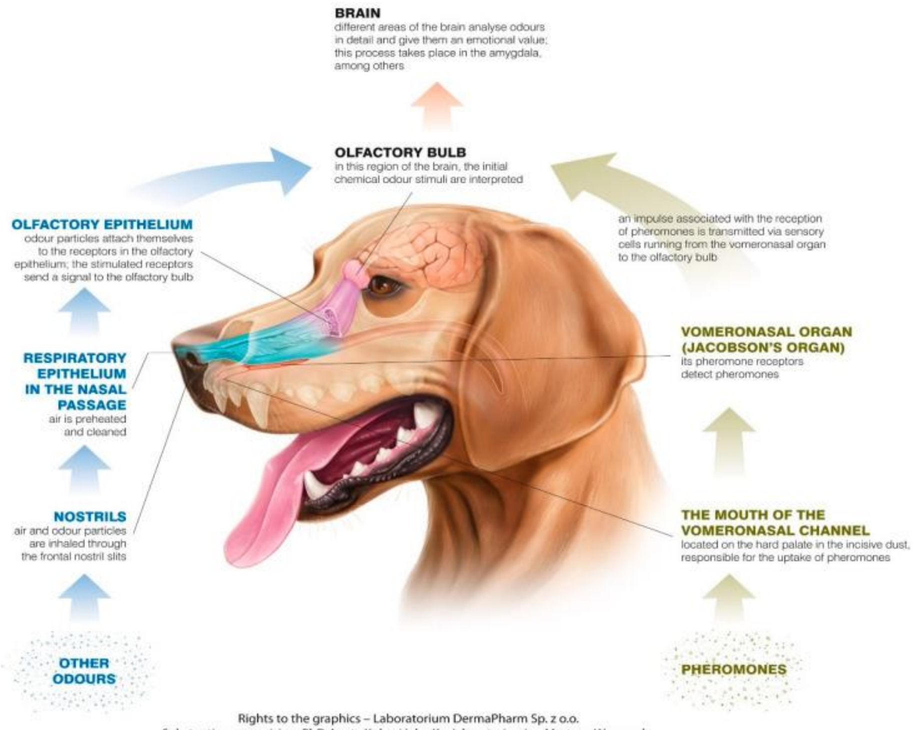

Figure 1: Schematic structure of the chemical substances detection system

3.2 What are detection dogs?

A detecting dog, also known as a sniffer dog, is a dog that is properly trained to detect a specific substance, by utilizing its highly developed sense of smell. This is accomplished by identifying the volatile organic compounds (VOCs) that are linked with the substance in question.

Humankind has recognized and harnessed the remarkable olfactory abilities of dogs for various fields, and this collaboration continues to evolve and expand. Dogs' sense of smell is estimated to be tens of thousands of times more sensitive than humans, giving them a unique advantage in a wide range of applications beyond traditional roles like hunting and herding. DD are considered to be reliable, resourceful, and cost-effective tools that provide significant benefits in various areas of expertise globally such as: security, forensic investigation, search and rescue, food safety, wildlife conservation, species detection, environment monitoring, medical detection and more.

Over the last several decades dogs have become an aid tool in numerous fields. In one of the significant groups of working, DD are trained to detect landmines and explosive materials. Today over 100 million abandoned landmines are scattered across the world; those are considered a hazard that can injure and potentially kill people. Thanks to detective canines, explosive chemicals and materials are identified and are handled appropriately by law enforcement. (Fjellanger, 2003).
Explosives can be recognized by DD sensing combustible odors, they are capable of differentiating it from other pyrolysis materials, and pinpointing the exact location of the flammable compounds. With DD the process of analysis is more precise and fewer samples are needed, in volumes as low as 5-000.5 µL, which is equal to the sensitivity of lab technique, therefore, saving time and money and improving the efficiency of the investigation. Working alongside the police force to help solve mysteries and crime and protect society from terrorists. (Agata, et al,2021).

Trained dogs are capable of tracing and identifying particles in quantities as low as 0.1 gram of chemicals across a large area, while other equipment have failed. Therefore, hazardous chemicals such as toluene and organochlorine pesticides like aldrin, dieldrin and DDT, can be detected and the DD can pinpoint the source for a more efficient sampling. In a study, a dog detected contaminated soil with less than 1 ppm with an accuracy of 99% (Crook, 2000).

Countless people across the globe at any given moment are subject to screening conducted by DD. These screenings are for the detection of illegal substances such as heroin, cocaine, methamphetamines, and others. These days it is one of the most common uses for DDs; they are used by customs services, police, and border control agents to identify illegal drugs in large and crowded areas such as airports, seaports, and even workplaces or schools. (Lorenzo, et al, 2003).

Biological detective dogs are used more frequently these days as well; they are trained to detect the scents of biological organisms and are able to recognize a specific odor connected to an organism and isolate it from the mixed smells of the environment. This trait is highly helpful and utilized by the police force to identify offenders. With the help of a dog's powerful olfactory system, they are able to connect a specific odor that has been found in a crime scene to a suspect. Furthermore, an average of 77.5% of trained dogs can trace the route of a specific person in a chaotic central area. (Harvey, 2003).

When it comes to search and rescue, canines trained to detect biological scents are an important asset in locating victims of natural disasters, earthquakes, plane crashes, avalanches, and floods, etc. Those trained canines, referred to as cadaver detection dogs or human remain detection dogs, possess the ability to detect decomposing human remains that may not be visible to the human eye. They can detect the residual odor of remains that are present in deep soils, on surfaces and in water sources even long after the remains have been removed. According to studies, those remarkable dogs have a success rate of over 75% in cases up to 667 days after the remains such as bones, body tissue and fluids, have been removed from the soil. (Michael, et al, 2015).

Biological safety and border control fields use trained canines to detect living animals. Dogs can detect with an effectiveness of 62% the brown tree snake, *Boiga irregularis,* which is considered an invasive species responsible for eradicating the majority of the native bird population in Guam. (Engeman, et al. 2002).
Parasitic insects, such as Palm weevil, affect the agriculture industry plantations of date palms in the Middle East; the specific odors that they secrete on affected trees are

identifiable by trained canines with a success rate of 73%.
Screw flies, which infect wounds on large animals and contaminate slugs, causing serious economic losses by damaging and killing warm-blooded animals, can be detected in 99.7% of cases by DDs. . (Browne, et al, 2006).
Eastern subterranean termites, *Reticulitermes hesperus Banks*, cause annual financial loss close to two billion dollars in damage and pest control in the USA. Usually, the early infestation stage is impossible to detect even by the expert eye, and in most cases is only visible when a damage has already been done. Special canines are trained to detect the insect in its early initial phase. A study has shown an accuracy of 98% while man-made special devices are not capable of detecting them at all. (Brooks, et al, 2003).

Microorganisms can also be detected by trained DD. A good example for that is the Cyanobacteria. This bacterium can be present on different fish species, causing an unpleasant odor on the flesh of the fish. This phenomenon is common in commercial fish farming. In the states alone, the loss can be up to 23 million dollars a year. Research has shown that trained dogs can identify the molecules of 2-methylisoborneol and geosmin within a water pond; these metabolites, produced by the cyanobacteria, are responsible for the foul taste and odor of the fish. Furthermore, canines are the perfect alternative method for analyzing chemical and unpleasant smells with a 79% to 92% accuracy. (Shelby, et al, 2004)
Dogs canrecognize mold, different fungi and other microorganisms that have a negative impact on human health and structural deterioration with a success rate of 75% . (Kauhanen, et al, 2002).

Conservation science fields use trained DD to discover endangered species in the wild; due to dense habitation, using traditional biological methods to keep track of such animals is often futile. Therefore, special "scat dogs" are trained to distinguish and track down the scats of endangered animals. This noninvasive technique helps experts analyze the found droppings and obtain data with the help of a molecular analysis machine, thus gathering information on species, sex, parasites, diet, the stress hormone, reproductive rate, and physical state of the organism. When this sampling data is gathered systematically over time, it can give the scientists a portrait of the endangered population.

Trained dogs have been used in North America for the security and assessment of the grizzly bears, *Ursus arctos horribilis,* population. The DD helps in protecting the game animals and in addition to humans. Canines trained to sense bear scats over an area of 500 km² help researchers identify each subject. (Wasser, et al, 2004).

Dogs in the USA that are trained to identify scats are used in research on a rare subspecies of kit fox called the San Joaquin, *Vulpes macrotis mutica.* However, identifying the fox's scats from similar droppings left by species such as the coyotes *Canis lurrans*, *skunks Mephitis mephitis*, and badgers *Taxidea taxus* poses a challenge to both dogs and humans. Astudy found that dogs were 100% accurate in identifying the fox,four times more successful than expert human trackers. This is valuable because DNA extraction from scats and laboratory methods for identifying species are expensive. (Smith, et al, 2003).

In Russia, dogs are trained to match urine samples collected in the wild to those in the data library to identify various species. Dogs are the traditional tracking methods used to monitor individual tigers' movements, and population dynamics. Study showed 89% and 96% accuracy in detecting tigers in a new study area. (Kerley, et al, 2007)

Dogs that were used for bird hunting in the past, are now being used to locate and study endangered bird species and in species management programs. For example, a Border collie was used to catch Aleutian cackling geese in order to relocate it to a predator free environment in Alaska. The dogs were far more efficient than humans; in just four days, two trained canines caught 143 birds whereas scientists only managed to catch 120 birds in the span of three weeks.
In New Zealand, dogs have been locating the endangered species of the Kiwi bird, *Apteryz spp*, the Kakapos, *Strigop habroptilus*, and Blue ducks, *Hymenolaimu malacorhynchos* for over 100 years. (Agata, et al,2021). (Dzie. et al,2013)

Dogs can detect changes in the reproductive cycle and differentiate between phases of heat in bitches. Furthermore, canines can recognize and detect odor change from vaginal fluid, urine, milk, and blood plasma of dairy cows in heat with an accuracy of 78% to 99%. They can also recognize the proestrus, estrus, and diestrus phases from milk odor. (kiddy, et al, 1984).

For the last two decades experts have been trying to utilize the dogs' olfactory abilities in the growing field of medicine. Dogs are trained to detect pathogens and diseases in humans and animals. They can smell the pathogen or detect the VOC that reflect the state of the metabolic organism. A dog trained to discover pathogens is called a biological detective dog, while a dog that detects the VOC that reflects the metabolic state is called a Medical biological detective dog.

These days trained dogs can recognize and alert people before medical episodes such, seizures, narcolepsy, hypoglycemic and more are going to happen, by sensing the change in body odor, exhaled air, urine, feces and other bodily fluids.

Trained canines are also capable of recognizing neoplastic changes in a patient's human as well as animal body. Additionally, trained dogs can detect different viral infections, parasites, bacteria and other pathogenic agents that can harm the health of a patient.

Figure 2: Detective dog smell tissue sample

4. Materials and methods

4.1 Obtaining materials and information.

During my thesis work, I conducted a comprehensive review of research articles published between the years 2000 and 2023 to find relevant scientific articles.

To ensure that I worked with reliable and objective materials and sources, I compared all the information I found with scientific literature and papers from academic sources, universities, and websites. For example, I first gathered broad information from online articles through a thorough web search. Then, I looked up the publisher(s) and sources of information and compared my latest findings with other more reliable or proven sources, which were mostly scientific literature in the form of books and research papers.

My primary sources of information were PubMed and Google Scholar for general scientific information about detection dogs and specific research on the topic. I used various keywords like "Biological detective dog", "Medical detective dog", "Detective dog", "Olfactory detection", "Dog olfactory", "Dog detective disease", and "Dog smell disease" to find the most recent and relevant articles.

In addition to that, I used various internet publications to gather information essential to this paper. I looked at different websites such as the American Kennel Club and World Health Organization.

Finally, I gathered relevant images from the articles I found to provide illustrations and a better understanding of the topic.

4.2 Analyzing research articles and studies.

The research scientific articles that I gathered are divided into different groups.
A few researches were systematic literature reviews of scent detection of cancer or other infectious diseases by dog, these articles were found at PubMed, Web of Science and Google Scholer on standard PRISMA- Reporting Items for Systematic Reviews and Meta-Analysis.

Some research articles used eNose based on a nanosensor array with gold nanoparticles (GNP), to compare and analyses the performance of the dog.

Most of the studies used different kinds of performance tests, training and evaluating methods of the trained canines. Most of the researches used the Double Blinded test; in this test neither the dog nor the trainer know the nature of the samples that should be analyzed. Other researches used the Single Blinded test, in which an operator that knows the nature of the samples is present and visible to the dog, while the trainer does not know the nature of the temple.

The raw result date summarizes to a sensitivity/ true positive- the trained dog probability indicates the correct Semple with intendent specific odor. And specificity/ true negative – the trained dog probability to disregard non disease sample. These sensitivity and specificity gave the research the general ability idea of trained dog.
The negative and positive results are calculated by using a biostatistics program and predicting the probability that the study would yield the correct diagnosis. Binomial probability from the result is used to compare the performance on the tested dog in a random selection of sample.

5. Results

5.1 Training dogs for medical purposes

Dogs are capable of detecting the elusive odor changes of volatile substances, they have the potential to become non invasive detectives or warning systems that the traditional diagnostic systems are not capable of providing just yet.

Most of the dogs that were used in studies reported to be dogs that have already been trained by professional dog handlers, and worked in the field of BDD. The dogs were trained to detect explosives, narcotics or r diseases. Most of the dogs worked with police forces, search and rescues, border controls, and fire fighters. Those dogs were selected because of their exceptional search pattern and their knowledge of how to indicate the specific odor. Therefore, the regular trainer/ handler of the dog was also used in the study to train the dog. This fact also contributes to the different dogs' methods of alerting of a positive sample, some sit, some stand beside the sample, some put their snot on the sample.

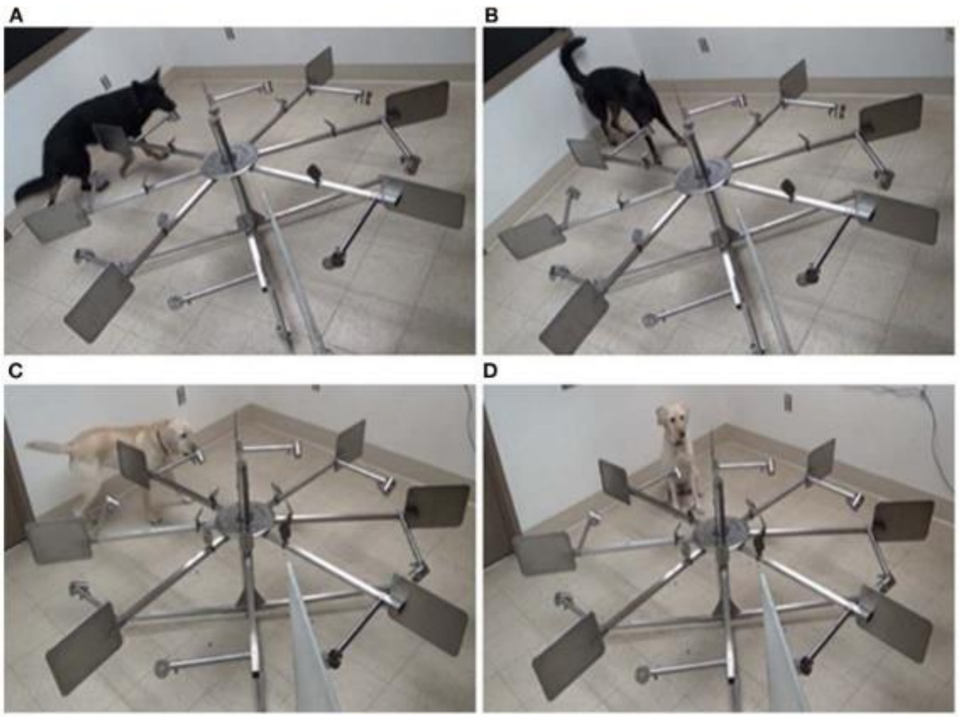

Figure 3: A-D: Detective dogs taking part in study.
A&C: detective dogs smelling samples in circle training aid device.
B&D: detective dog alert the handler they found the positive sample,
The black DD alert by putting his snot on the instrument
The white DD alert by sit in front of the senple.

The studies conducted focused on two types of conditioning- classical conditioning and operant conditioning. Positive reinforcement was used as the primary method for training, with rewards in the form of food or toys as determined by the trainer. Clicker training was also used, which involves creating an association between the sound of a clicker and the primary reinforcer reward, thereby making the clicker a secondary reinforcer.

The number of trained DD used per study varies between 1 to 10. The training periods that have been observed within studies varied from a few weeks as the case of McCulloch, et al 2006, and up to 5 years in Sonoda, et al,2011. Depending on the research team and the complexity of the exercise, the occurrence of the training extends from once a week to a few sessions per day.
This difference between the studies could be connected to the type of the researchable diseases, the variance between patients and controls, the different body samples, the quality of samples, training differences or the animal's abilities.

The training protocol consists of three stages. In the first stage, the trained dog learns to identify a specific smell in the positive sample, which is obtained from cultures, urine, sweat, saliva, etc., and placed in a jar or box training aid device.

In the second stage, the trained canine is exposed to both the positive and samples that do not contain the specific odor - the negative / control samples, which are present in a training aid device but not at the same time. This stage usually takes longer than the others.

The third stage is the experiment stage, where the trained dog's response rate to positive, negative samples and in some studies distractor samples, which are not positive or control samples. Those samples were tested in the same room.

Figure 4: lined training aid device tool

The number of samples used in the training aid device ranges from 2 to 12 and can be presented in lines, circles, or randomly.

The line method is advantageous when designing a "blank" run, where all the samples are negative. At the end of the row, the DD can indicate that no positive sample was found. The circle presentation can also be used for a blank run, where all the samples are equal, and there is no beginning or end.

Keeping a space between the samples is necessary to prevent cross-contamination and correctly stimulate the olfactory sensation. Dogs that are trained to become medical DD are usually already trained as BDD.

The studies used different methods of blinding condition:
Unblinded condition: the dog handler knows where the positive sample is.
Single blinded condition: only the research operator that is present in the room knows where the positive sample is, the dog handler does not know.
Double – blinded condition: no one in the room knows where the positive sample is. Only someone who is watching outside knows where the correct sample is.

Training detection dogs has several advantages, including their noninvasive and fast nature, as well as cost-effectiveness compared to existing detection methods. However, the accuracy of detection dogs can be significantly impacted by extraneous odors. Additionally, the disadvantage is associated with their reliance on handlers, as well as physiological factors such as exhaustion. Furthermore, a lack of research in the field can also impact the results.

Selecting the sample training aid, a very important decision, can affect the dog's ability to recognize the pathogen.
Odor absorption-based training aids, a safe method to acquire and discharge the selected odors. Those substrates absorbed the odor of the pathogen or the disease state. The based training aids have many variants, one of them is PMDS.
Polydimethylsiloxane based training aid is a polymer based adsorption that was used for explosives at first. This is a non-toxic, non-infectious based training aid that can be soaked with almost every odor.

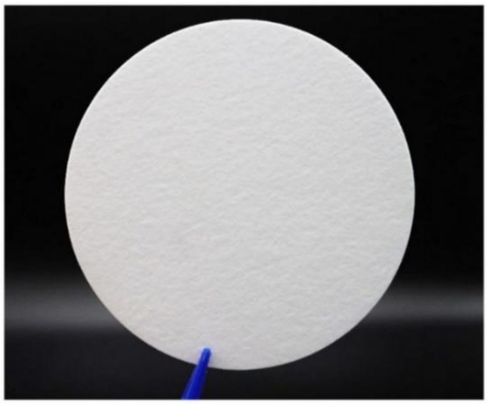

Figure 5: Cellulose microfiber-based filter paper for odor soaked particle

Figure 6: cotton swab soaked with breath particles sample in glass tube.

Getxent tube, is another example for an odor absorption aid used in canine training. This is a small diameter tube, containing polar and non-polar sections that contribute to the absorption, storage and specific targeted VOC release. These tubular tool aids were used extensively during the Corona pandemic and research phase of COVID-19 patients.

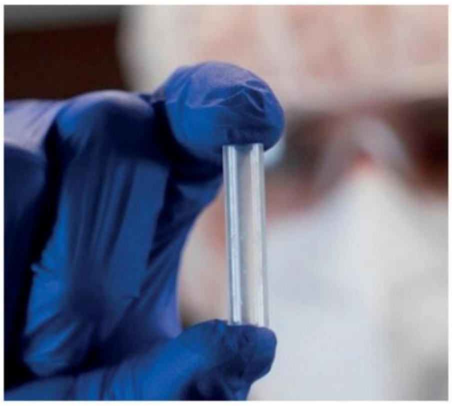

Figure 7: getxent odor adsorption tube training aid tool

Traditional odor soaks – one of the oldest aid tools, used in the narcotics, explosive and many other fields of DD. The targeted odor is impregnated in a sterile laboratory environment on cellulose or microfiber paper.

In an odor sample that is connected to infectious diseases or can harm the MBDD, a measure of safety is taken into account. A filter barrier is placed between the sample and the odor soak substrate, to ensure no accidental contamination of the sample or potential hazards to the dog.

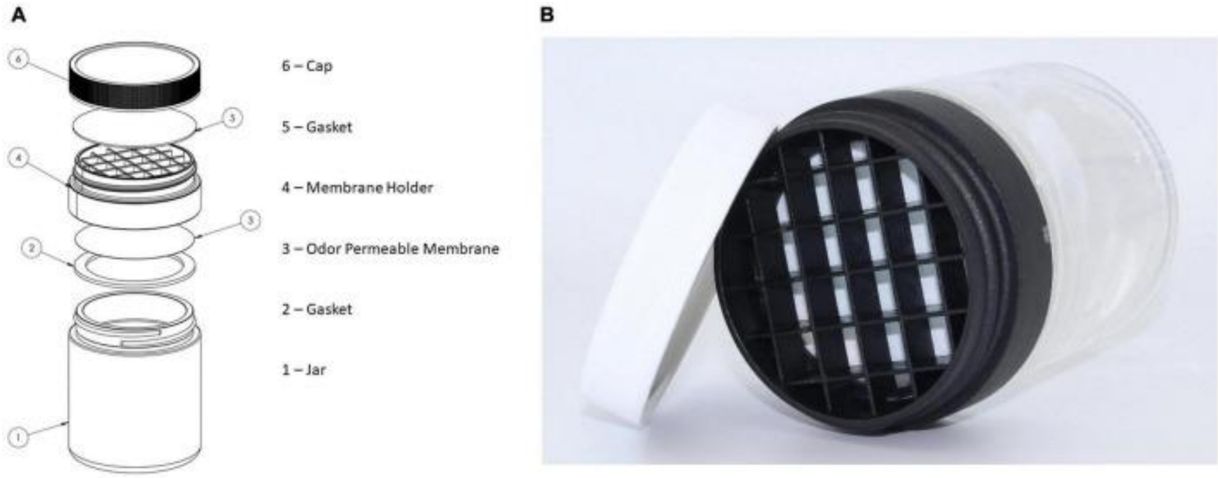

Figure 8: Training aid delivery device sample with proper safety protection and cross contamination.
A: breakout diagram
B: photograph

5.2 Which dog breeds and crossbreeds can be used?

Dogs have a highly developed sense of smell that has been extensively studied and used in various fields of investigation. When it comes to detecting human diseases, dogs are often the first choice. They can be trained for specific targets and are available all over the world.

However, there is limited information on how dogs are selected for human research, other than their sense of odor and motivation. Certain dog breeds and crossbreeds are more effective for detection work than others, particularly those that were originally bred and specialized as work breeds for decades. Brachycephalic dogs are not ideal for olfactory performance due to their short-nosed anatomical structure, which negatively impacts air movement through their nasal passages, making them less effective at detecting odors (Polgar et al., 2016).

Currently, the information regarding the olfactory capacity of different breeds is inconsistent. Jezierski et al. (2014) found that German Shepherds were considerably more reliable than Labradors and Terriers in detecting narcotics drugs, while Hall et al. (2015) found that Pugs were better at tasks involving olfactory perception and achievement tasks, compared to German Shepherds and Greyhounds.

The most common canine breeds used in detecting disease odors are Malinois, German Shepherds, and Labrador Retrievers, as they are considered more reliable in detecting odors. Other breeds used in some studies include Cocker Spaniels, Springer Spaniels, Golden Retrievers, and Beagles. In one study, a crossbreed between a Dachshund and a Shepherd was used, and in another, a crossbreed between a Labrador Retriever and a Pitbull.

Overall, the choice of trained dogs mainly depends on their trainability and willingness to learn, as well as their performance and favorites by their trainers. Experienced sniffing dogs may be beneficial for mastering new tasks, but there is no general proposal. The most important idea is that the trainer will know and understand the dog's behavior.

5.3 Which diseases can be diagnosed by a detection dog?

The first case report of a dog recognizing a disease was published in 1989, when a dog's owner was concerned about his excessive sniffing behavior. The pet obsessively smelled a suspicious mole on the owner's body, that later on was determined to be melanoma. (Williams and Pembroke, 1989).

Since that observation BMDD trained to recognize different diseases in exhaled air, feces, urine, and other tissue samples.

Three different studies, conducted in 2012, 2017 and 2018, Taylor at el, proved that DD can identify Clostridum *difficile* from cultures and stool samples. This spore forms a bacterial nosocomial infection that is transmitted from the environment or from contaminated hands. The organism can survive in the environment for a long time, causing symptoms varying from mild diarrhea to fulminant pseudomembranous colitis. In the USA alone there are 450,000 estimated cases per year.

Koviusalo's research in 2017, proved that canines can detect VOC of Meticillin – resistant Staphylococcus aureus. This Gram-positive bacterium is resistant to beta lactam antibiotics, causing severe infection with increased morbidity and mortality. Its unique ability can cause more than 100,000 cases of deaths around the world every year.

A study by Maurer in 2016 confirmed dogs' ability to alert to urinary tract infections, caused by E. *coli*, Enterococcus, Klebsiella and S.*aureus* bacterias. This significant medical problem which can lead to life-threatening complications, can be detected by DD from a simple noninvasive urine sample.

A pilot study focusing on lung damage in Cystic fibrosis patients with infection caused by Pseudomonas *aeruginosa*, a Gram-negative bacterium infecting 60% of CF patients, found that the bacterium can be confirmed and distinguished by trained DD from other opportunistic bacteria encountered in CF. (Davies, et al, 2019).

In 2019, a study showed that dogs can detect Malaria parasites from the foot odor of Gambian children ages 5 – 13 years old. (Guest, et al, 2019). Malaria is a severe and, in some cases, life-threatening disease in humans. Caused by five species of Plasmodium parasites. P. *falciparum* and P. *vivax* pose the most significant threat. P. *falciparum* is the deadliest malaria parasite and is most prevalent in Africa, while P. *vivax* is the most common species outside of sub-Saharan Africa. The other species that can infect humans include P. *malariae*, P. *ovale*, and P. *knowlesi*. The parasite is mostly transmitted to humans by infected female Anopheles mosquitoes. However, it can also be transmitted through blood transfusions and contaminated needles. The estimated number of deaths from malaria in 2021 was 619,000 people, nearly four out of five were children under five years old, mostly in Africa.

Haedin, in 2015, proved that trained dogs can detect hypoglycemia samples from patientד with type 1 diabetes. Hypoglycemia commonly occurs as a side effect of insulin injection; left untreated it can lead to an increase in morbidity and mortality. Canine can be successfully trained to detect hypoglycemic episodes through a patient's breath and can be companion diabetes alert dogs. (Reeve, et al, 2020).

Epilepsy is a neurological condition that affects around 65 million people around the globe, and it can be severely debilitating and even life-threatening. Unfortunately, currently there is no simple and reliable device that can warn patients of an impending seizure, causing those with unstable epilepsy to live in constant fear of sudden injury or death. (powell, et al, 2021). Catala proved in 2019 that medical DD can recognize and alert of incoming seizures. Furthermore, as part of subsequent experiments in 2020, Catala confirmed that trained DD can sense VOC associated with pre seizure. a study in 2021 demonstrated the ability of trained dogs to predict seizures up to 90 minutes preceding the event. (Maa, et al,2021).

Obstructive sleep apnea is considered to be the most common sleep related breathing disorder, described as recurrent events of complete or partial obstruction of the upper airway, causing reduced or absent breathing through sleep. Koskinen proved in 2019 that trained dogs can sense and recognize and distinguish people with OSA by urine samples based on a specific odor.

Melanoma is a form of cancer that originates from the pigment-producing cells known as melanocytes. While those tumors usually develop on the skin, they can occasionally occur in other parts of the body such as the mouth, intestines, or uveal melanoma. In women, melanomas usually appear on the legs, whereas in men, they tend to occur on the back. Pickel et al, 2004 first proved trained DD can distinguish melanoma from different tissue samples. Furthermore, Walcazak et al, in 2012 showed trained DD's ability to recognize melanoma from a breath sample in 2016 Willis et al, proved that canines can recognize melanoma cells in vivo, on patients' skin.

IN 2004 Willis et al, proved his hypothesis that trained sniffer dogs can recognize bladder cancer cells from urine samples. Moreover, in 2011 they proved other evidence that VOC biomarkers odor connected with bladder cancer in urine samples can help with diagnosing this type of tumor that develops from the epithelial cells that are lining the urinary bladder.

Guerrero - Flores et al in 2017, said that cervical cancer nowadays has become a public health concern among females worldwide especially in developed countries with low income population sectors. An alarming example can be seen in Mexico with 15.5% of cancer cases and 12.8% mortality in the female population. A study suggested trained dogs as a non-invasive, affordable and accessible tool to detect cervical cancer cells odor in patient tissue biopsies, smear samples and surgical bandages that have been used as intimate sanitary pads. A pilot study in 2020 demonstrated successfully distinguish cervical cancer and cervical intraepithelial neoplasia grade 3 from urine sample by trained sniffer dog. (Yamamoto, et al, 2020).

The incidence of ovarian carcinoma is around 4% in cancers diagnosed in women and because of late diagnosis the mortality rate is above 50%. Horvath et al in 2008, proved that trained dogs can distinguish different histopathological types and category grades of ovarian carcinoma. The researchers also proved in 2010 that dogs can also detect the ovarian carcinoma in blood plasma samples from patients. Furthermore, they confirmed that a specific marker's odor is connected to a specific type of cancer and can be distinguished with the help of sniffer trained dogs. In 2019 Murarka et al, suggested DD should be trained on cancer cell line odor of OVCAR-8, SK-OV-3, and OVCAR-4, to contribute to the odor detection of the sniffer dog, lower cost, lesser risk of infection in patients and more sample availability.

In 2006 McCulloch et al confirmed that trained DD can remarkably detect all 4 stages of breast cancer from breath samples. Breast cancer is the second most common cause of death mainly in women worldwide. In 2018 Seo et al, cultivated cancer cell culture with mammary carcinoma cells – 4T1, have been detected by trained canines. Thuleau et al in 2019 proved the non-invasive transcutaneous method to detect breast cancer by utilizing trained sniffer DD to smell sweat Samples from medical sterile pads that had been on patients' breast overnight. Furthermore in 2021 a new potential screening method based on the ability of trained DD to recognize the specific VOCs in urine samples was proven. (Kure, et al, 2021).

Lung cancer detection using trained DD has been extensively researched since 2006, with over 15 studies conducted in the field. Most of them used dogs to detect the cancer from breath samples. In 2019 Feil, at al, proved that DD can also recognizelung cancer from urine samples, and Junqueira et al proved detection from blood serum. In 2023 Lie provided evidence that dogs recognizing lung cancer from breath samples have a higher sensitivity and specificity than urine samples. Furthermore, they have a100% diagnostic rate in 1A stage lung cancer.

Colorectal cancer is the third most common cancer and accounts for around 10% of all cancer cases globally and is the second leading cause of cancer-related deaths worldwide. In 2020, a pilot study conducted by Schoon et al, demonstrated that trained dogs can detect the presence of colorectal cancer from stool odor samples. This study highlights the potential of using dogs as a non-invasive and cost-effective means of detecting cancer.

Prostate cancer is the second most common and leading cause of death in men in the developed world. Cornu et al, 2011, shows that trained canines can identify VOC of prostate cancer in urine samples. Furthermore, In 2021 Gusest et al, proposed to use trained dogs as a more sensitive and specific detection alternative strategy for prostate cancer, beside the current serum prostate specific antigen screening.

COVID-19 is a highly contagious disease caused by the SARS-CoV-2 virus. The disease was first identified in Wuhan, China in December 2019 and has since then has spread globally, resulting in the COVID-19 pandemic, which claimed the lives of nearly 7,000,000 people. Researchers from around the world are working to find a reliable method for detecting COVID-19, and one of the most promising areas of study currently is the use of trained dogs and their ability to detect the virus. Over the past three years, there have been more than 30 studies conducted on this topic. Jendrny et al. (2021) have successfully proved that COVID-19 can be detected by trained dogs using body fluid samples. Additionally, Hagen et al. (2021) have shown that trained dogs can differentiate SARS-CoV-2 infections from other respiratory infections, while Hag-Ali et al. (2021) have demonstrated that the sensitivity of trained dogs is greater than that of the gold-standard RT-PCR test.

5.4 Effectivity of canine smell detection

Medical detection dogs are specially trained dogs that have the ability to detect various diseases in different types of samples such as feces, urine, blood, plasma, tissue, cell culture, and other body fluids. In some studies, even when the sample had a low VOC concentration, dogs werestill able to detect the status of the disease.

Some researches have shown very promising results, such as the study by Sonoda, et al, 2011, which indicated that dogs can detect patients with colon cancer in their breath and stool with a sensitivity and specificity of 0.91 and 0.99, respectively, from the breath sample. In addition to that, the sensitivity and specificity from stool samples were 0.97 and 0.99, respectively.

In another study by Bomers, et al, 2014, Clostridium difficile was identified in 12 out of 14 stool samples, with a sensitivity of 86% and specificity of 97%, indicating 346 out of 357 negative samples.

The ovarian cancer detection test showed a high sensitivity of 97% and specificity of 99%, detecting 41 out of 42 positive Samplings, and only 2 out of 210 control samples were not recognized, for the patients' plasma, according to Horvath, et al, 2013.

Among 55 lung cancer patients, the overall sensitivity of canine detection compared to biopsy-confirmed conventional diagnosis was 0.99, and the overall specificity was 0.99. Among 31 breast cancer patients and controls, sensitivity was 0.88 and specificity was 0.98. Those results of sensitivity and specificity were remarkably similar across all four stages of both diseases, as reported by McCulloch, et al, 2006.

Prostate cancer detection results with two dogs showed that dog 1 had a sensitivity of 100% and specificity of 98.7%. In addition, dog 2 had a sensitivity of 98.6% and specificity of 97.6%, according to Taverna, et al, 2015.

In a study by Mauren et al, 2016, samples were obtained from 687 individuals who had UTI, and dogs detected 250 urine samples positive for Escherichia coli with a sensitivity of 99.6%, and a specificity of 91.5%. Diagnostic accuracy was similar to

50 samples of Enterococcus with a sensitivity of 100% and specificity of 93.9%.
50 samples of Klebsiella with 100% sensitivity and 95.1% specificity.
And from 50 samples of Staphylococcus aureus, the sensitivity was 100%, specificity 96.3% . All dogs performed with similar accuracy: overall sensitivity was at or near 100%, and specificity was above 90%.

The sensitivity and specificity of disease detection varied greatly across studies, with some achieving perfect rates while others performed at chance level. Additionally, there was considerable variation among studies examining the same disease, using different body sample matrices and detectors. Some publications reported low sensitivities of 0.17 (Gordon et al) and specificities around 0.29 (Amundsen et al), while others achieved rates of 1 in sensitivity (Horvath et al, Cornu et al, Sonoda et al) and specificity.

5.5 Factors affecting canine medical detective.

As any biological system, detective dogs' olfactory sense can be affected by internal physiological and external factors.

Initially and maybe the most critical one is the genetic factor, canine OR combine 1094 genes, three times more compared to humans. This date is believed to be the direct result of the broad range of canine capabilities of smells. 50% of OR genes are considered to be pseudogenes in humans, while in dogs only 20% of RO are functioning. The percentage of the pseudogenes gene combined with the gene polymorphism is considered to vary between the species and the breed. Those differences result in the olfactory capability variation among the individual canine. (Jenkins, et al, 2018).

Currently, the largest group of genes known in mammals is the OR genes, which have a wide range of versatility within canine breeds. That can affect their olfactory abilities. Research has found that there is gene polymorphism expressed in the olfactory receptors, with breed-specific alleles. (Tacher, et al,2005).

Nevertheless, different research tries to establish which dog breed is the most sensitive to olfactory. The results were inconclusive and indicated that in addition to genetics, behavioral elements needs to be taken into consideration such as the ability to work with a trainer and motivation and willingness to learn. Those factors could have an important impact on olfactory capabilities. Furthermore, the behavior component is equally important as the genetic one in determining the olfactory capabilities. (Agata, et al,2021).

As with any living organism, time and age have an impact on its abilities. Olfactory capabilities decrease with age; it has been observed that dogs from the age of 14 years old suffer from atrophic in the olfactory epithelium. And over the age of 17, the deterioration is prominent. (Hirai, et al, 1996).

Older dogs, compared to juvenile canines, present strong long-term memory of smell, which contribute to their ability to detect and analyze complex odor information.

Sex differences also play a role in demonstrating olfactory abilities. Research has shown that female dogs demonstrate a stronger utilization of their sense of smell. This is proven by detecting a larger number of active cells in the OB in bitches, compared to male dogs. This result may be nativity connected to the fact female dogs need to find food for their puppies (Wei, et al, 2017).

Environmental conditions can impact the canine olfactory abilities as well. Humidity, for example, has a direct effect on odor analysis; there is a correlation between improving nasal humidity and trapping the odor molecules, together with the barometric pressure. Research proved that trained dogs performed better in relatively high-humidity climates due to increasing odor particle intensity. On the other hand, rainy weather or significantly high humidity decreas residual odor. (Jenkins, et al, 2018).

The combination of humidity and fog badly influences the olfaction, resulting in the odor particles being suspended in the air and forcing the trained dog to process the entire area, affecting the time it takes to track the source.

Another negative influence can be attributed to high temperatures, causing poor searching performance, directly affecting the dog's body, with the risk of dehydration. (Bräuer and Blasi, 2021).

Olfactory activity can also be affected by the nutritional elements that are present in the canine’s diet. Corn oil and animal-based protein can increase olfactory performance, while coconut oil decreases the activity.

Diseases that cause improper airflow also affect the olfactory abilities. Illnesses such as nasal cavity toners, injuries, viruses, and bacterial infections such as parainfluenza or canine distemper are some of the causes.

Hyposmia is a decreased sense of smell caused by endocrinological diseases such as hypothyroidism, hyperadrenocorticism, diabetes, other neural mechanisms, excess mucosa production, neoplasia, etc. Hyposmia type II is the decreased ability to recognize odor particles, this phenomenon can cause DD to fail to achieve their goal. (Jenkins, et al, 2018).

Drugs can also have an influence on the sense of smell and even cause loss of function or temporary dysosmia, usually as a result of inhibition OR.

There are a few studies that show the connection between drugs and their effect on the canine olfactory. Among the research, Jenkins et al. noticed that Metronidazole as an oral administration decreases the ability of trained canines to detect the smell of explosives. The use of steroids such as Dexamethasone or Hydrocortisone causes an increase in the olfactory detection threshold.

Sampling protocols of body material play a key role in a proper quality sample. If the sampling is not supervised, or not properly done, it has a risk of affecting the results of the study. In addition to that, if the samples are not properly kept it also can cause cross-contamination and change the study results.

As all research has shown, the ability of the dog is influenced also by the educational environment, the knowledge of the trainer, and the capacity of the dog to learn. More than that, a correct training program should be used, that includes accurate odor samples without contamination.

6. Conclusion and discussion

The purpose of this literature review is to explore the concept of using dogs as detection animals. Dogs have been used for centuries due to their remarkable sense of smell, which is a result of their unique anatomy and physiology.

In today's world, dogs are trained to develop their sense of smell and are used in various fields such as explosive detection, law enforcement, search and rescue, biological surveys, and more.

In the past 30 years, a new trend has emerged where dogs are trained to detect volatile organic compounds even from the smallest samples for medical purposes. These medical detective dogs can detect different bacteria, viruses, diseases, and illnesses from body fluid samples. This offers a new optional method that has many advantages as it is mobile, non-invasive, provides real-time detection results, and is cost-effective. Furthermore, in some cases, it can alert earlier than traditional screening methods which can increase the survival range.

Canine detection appears to be more promising for infectious diseases rather than non-infectious diseases like cancer, diabetes mellitus, and epileptic seizures. Despite some initially promising studies, published data can vary significantly for the identification of cancer. Studies have achieved very different results with a broad range of sensitivity and specificity in the identification of different cancer types such as bladder, prostate, or ovarian cancer, lung and breast cancer, as well as colorectal neoplasms. This suggests that different factors can affect dogs' capability to detect, such as breed, sample preparation, training, and other elements. Therefore, further research is needed to explore the full potential of medical trained dogs.

7. Summary

It is widely known that dogs are not only man's best friend, but they are also highly efficient odor detectors. Thanks to their incredible olfactory system, they can sense and locate an extensive range of odors in a large area. This outstanding ability, combined with their trainability and intelligence, makes them excellent detective dogs. In recent years, they have played a significant role in many fields of expertise, including medical detective dogs.

Medical detective dogs have proven to be valuable assets in the medical field. They can detect illnesses and diseases in a shorter amount of time, are cost-efficient, non-invasive, and more reliable than some of the existing tools and methods. This allows people to be diagnosed in time, get treated and increases their chances of survival.

However, despite their high sensitivity and specificity, there are still limitations that affect their detection abilities. Further research should be conducted to improve their efficiency and accuracy.

Acknowledgements

I would like to thank my supervisor, Professor László Zöldág with the Institute for Animal breeding, Nutrition and Laboratory Animal Science, Department for Veterinary Genetics and Animal Breeding for allowing me this interesting thesis subject.

Author,
Avigail Sala Simon

8. list of references

1. A.E. Juge, M.F. Foster, C.L. Daigle Canine olfaction as a disease detection technology: a systematic review Appl. Anim. Behav. Sci., 253 (2022), Article 105664
2. Bauër P, Leemans M, Audureau E, Gilbert C, Armal C, Fromantin I. Remote Medical Scent Detection of Cancer and Infectious Diseases With Dogs and Rats: A Systematic Review. Integr Cancer Ther. 2022 Jan-Dec;21
3. Biehl W, Hattesohl A, Jörres RA, et al. VOC pattern recognition of lung cancer: a comparative evaluation of different dog- and eNose-based strategies using different sampling materials. Acta Oncol. 2019;58:1216-1224
4. Bomers M K, van Agtmael M A, Luik H, van Veen M C, Vandenbroucke-Grauls C M J E, Smulders Y M et al. Using a dog's superior olfactory sensitivity to identify Clostridium difficile in stools and patients: proof of principle study BMJ 2012
5. Bräuer, J.; Blasi, D. Dogs display owner-specific expectations based on olfaction. Sci. Rep. 2021, 11, 1–10
6. Brooks, S.E.; Oi, F.M.; Koehler, P.G. Ability of Canine Termite Detectors to Locate Live Termites and Discriminate Them from Non-Termite Material. J. Econ. Entomol. 2003, 96, 1259–1266. [CrossRef]
7. Bryce E, Zurberg T, Zurberg M, Shajari S, Roscoe D. Identifying environmental reservoirs of Clostridium difficile with a scent detection dog: preliminary evaluation. *J Hosp Infect*. 2017,97(2):140-145.
8. Buszewski B, Ligor T, Jezierski T, Wenda-Piesik A, Walczak M, Rudnicka J. Identification of volatile lung cancer markers by gas chromatography-mass spectrometry: comparison with discrimination by canines. Anal Bioanal Chem. 2012;404:141-146
9. Catala A., Grandgeorge M., Schaff J.-L., Cousillas H., Hausberger M., Cattet J. Dogs demonstrate the existence of an epileptic seizure odour in humans. *Sci. Rep.* 2019;9:4103.

10. Catala A., Latour P., Martinez-Caja A.M., Cousillas H., Hausberger M., Grandgeorge M. Is there a Profile of Spontaneous Seizure-Alert Pet Dogs? A Survey of French People with Epilepsy. *Animals.* 2020;10:254.
11. Cornu JN, Cancel-Tassin G, Ondet V, Girardet C, Cussenot O. Olfactory detection of prostate cancer by dogs sniffing urine: A step forward in early diagnosis. Eur Urol. 2011
12. Crook, A. Use of odour detection dogs in residue management programs. Asian-Australas. J. Anim. Sci. 2000, 13, 219.
13. Davies JC, Alton E, Simbo A, et al. Training dogs to differentiate Pseudomonas aeruginosa from other cystic fibrosis bacterial pathogens: not to be sniffed at?. Eur Respir J. 2019;54
14. Dzie˛cioł, M.; Niz˙an´ ski,W.; Stan´ czyk, E.; Kozdrowski, R.; Najder-Kozdrowska, L.; Twardon´ , J. The influence of antibiotic treatment of bitches in oestrus on their attractiveness to males during mating. Pol. J. Veter. Sci. 2013, 16, 509–516.
15. Engeman, R.M.; Vice, D.S.; York, D.; Gruver, K.S. Sustained evaluation of the effectiveness of detector dogs for locating brown tree snakes in cargo outbound from Guam. Int. Biodeterior. Biodegrad. 2002, 49, 101–106.
16. Feil C, Stein T, Forster A, et al. Diagnosis of lung cancer by canine olfactory detection in urine and breath samples. J Clin Oncol. 2019
17. Fjellanger, R. The REST Concept. In Mine Detection Dogs Training, Operations and Odour Detection; McLea, I.G., Ed.; Geneva International Centre for Humanitarian Demining (GICHD): Geneva, Switzerland, 2003; pp. 53–105.
18. Gordon RT, Schatz CB, Myers LJ, et al. The use of canines in the detection of human cancers. J Altern Complement Med. 2008;14:61-67.
19. Guerrero-Flores H, Apresa-García T, Garay-Villar, et al. A non-invasive tool for detecting cervical cancer odor by trained scent dogs. BMC Cancer. 2017;17:79.
20. Guest C, Harris R, Sfanos KS, et al. Feasibility of integrating canine olfaction with chemical and microbial profiling of urine to detect lethal prostate cancer. PLoS One. 2021;16:e0245530
21. Guest C, Pinder M, Doggett M, Squires C, Affara M, Kandeh B, Dewhirst S, Morant SV, D'Alessandro U, Logan JG, Lindsay SW. Trained dogs identify

people with malaria parasites by their odour. Lancet Infect Dis. 2019 Jun;19(6):578-580

22. Guirao A, Molins L, Ramón I, et al. Trained dogs can identify malignant solitary pulmonary nodules in exhaled gas. Lung Cancer. 2019;135:230-233
23. Hag-Ali M, AlShamsi AS, Boeijen L, Mahmmod Y, Manzoor R, Rutten H, et al. The detection dogs test is more sensitive than real-time PCR in screening for SARS-CoV-2. Commun Biol. (2021) 4:2232
24. Hardin DS, Anderson W, Cattet J. Dogs Can Be Successfully Trained to Alert to Hypoglycemia Samples from Patients with Type 1 Diabetes. Diabetes Ther. 2015 Dec;6(4):509-517
25. Harvey, L.M.; Harvey, J.W. Reliability of bloodhounds in criminal investigations. J. Forensic Sci. 2003, 48, 2002118.
26. Hirai, T.; Kojima, S.; Shimada, A.; Umemura, T.; Sakai, M.; Itakurat, C. Age-related changes in the olfactory system of dogs. Neuropathol. Appl. Neurobiol. 1996, 22, 531–539.
27. Horvath G, Andersson H, Nemes S. Cancer odor in the blood of ovarian cancer patients: a retrospective study of detection by dogs during treatment, 3 and 6 months afterward. BMC Cancer. 2013;13:396
28. Horvath G, Andersson H, Paulsson G. Characteristic odour in the blood reveals ovarian carcinoma. BMC Cancer. 2010;10:643.
29. Horvath G, Järverud GA, Järverud S, Horváth I. Human ovarian carcinomas detected by specific odor. Integr Cancer Ther. 2008;7:76-80.
30. Jendrny P, Twele F, Meller S, Schulz C, von Köckritz-Blickwede M, Osterhaus ADME, et al. Scent dog identification of SARS-CoV-2 infections in different body fluids. BMC Infect Dis. (2021) 21:707
31. Jenkins, E.K.; DeChant, M.T.; Perry, E.B. When the Nose Doesn't Know: Canine Olfactory Function Associated with Health, Management, and Potential Links to Microbiota. Front. Veter. Sci. 2018, 5, 56
32. Junqueira H, Quinn TA, Biringer R, et al. Accuracy of canine scent detection of Non-Small cell lung cancer in blood serum. J Am Osteopath Assoc. 2019;119:413
33. Kauhanen, E.; Harri, M.; Nevalainen, A.; Nevalainen, T. Validity of detection of microbial growth in buildings by trained dogs.Environ. Int. 2002, 28, 153–157.

34. Kerley, L.L.; Salkina, G.P. Using Scent-Matching Dogs to Identify Individual Amur Tigers from Scats. J. Wildl. Manag. 2007, 71,1349–1356.
35. Kiddy, C.; Mitchell, D.; Hawk, H. Estrus-Related Odors in Body Fluids of Dairy Cows. J. Dairy Sci. 1984, 67, 388–391.
36. Koivusalo M, Vermeiren C, Yuen J, Reeve C, Gadbois S, Katz K. Canine scent detection as a tool to distinguish meticillin-resistant Staphylococcus aureus. *J Hosp Infect*. 2017;96(1):93-95.
37. Kokocińska-Kusiak A, Woszczyło M, Zybala M, Maciocha J, Barłowska K, Dzięcioł M. Canine Olfaction: Physiology, Behavior, and Possibilities for Practical Applications. Animals (Basel). 2021 Aug 21;11(8):2463.

Lippi, Giuseppe and Heaney, Liam M.. "The “olfactory fingerprint”: can diagnostics be improved by combining canine and digital noses?" *Clinical Chemistry and Laboratory Medicine (CCLM)*, vol. 58, no. 6, 2020, pp. 958-967.

38. Koskinen, A., Bachour, A., Vaarno, J. *et al.* A detection dog for obstructive sleep apnea. *Sleep Breath* **23**, 281–285 (2019).
39. Kure S, Iida S, Yamada M, et al. Breast cancer detection from a urine sample by dog sniffing. Biology. 2021;10:517
40. Liu SF, Lu HI, Chi WL, Liu GH, Kuo HC. Sniffer Dogs Diagnose Lung Cancer by Recognition of Exhaled Gases: Using Breathing Target Samples to Train Dogs Has a Higher Diagnostic Rate Than Using Lung Cancer Tissue Samples or Urine Samples. Cancers (Basel). 2023;15(4):1234
41. Lorenzo, N.; Wan, T.; Harper, R.J.; Hsu, Y.-L.; Chow, M.; Rose, S.; Furton, K.G. Laboratory and field experiments used to identify Canis lupus var. familiaris active odor signature chemicals from drugs, explosives, and humans. Anal. Bioanal. Chem. 2003, 376, 1212–1224.
42. M.B. Alexander, T.K. Hodges, J. Bytheway, J.A. Aitkenhead-Peterson application of soil in forensic science: residual odor and HRD dogsForensic Sci. Int., 249 (2015), pp. 304-313
43. Maa E., Arnold J., Ninedorf K., Olsen H. Canine Olfaction of volatile organic compounds unique to epileptic seizure. *Epilepsy Behav.* 2021;115:107690.

44. Maughan MN, Best EM, Gadberry JD, Sharpes CE, Evans KL, Chue CC, Nolan PL, Buckley PE. The Use and Potential of Biomedical Detection Dogs During a Disease Outbreak. Front Med (Lausanne). 2022 Apr 4;9:848090.
45. Maureen T Taylor, Janine McCready, George Broukhanski, Sakshi Kirpalaney, Haydon Lutz, Jeff Powis, Using Dog Scent Detection as a Point-of-Care Tool to Identify Toxigenic *Clostridium difficile* in Stool, *Open Forum Infectious Diseases*, Volume 5, Issue 8, August 2018.
46. Maurer M, McCulloch M, Willey AM, Hirsch W, Dewey D. Detection of Bacteriuria by Canine Olfaction. Open Forum Infect Dis. 2016 Mar 9;3(2)
47. McCulloch M, Jezierski T, Broffman M, Hubbard A, Turner K, Janecki T. Diagnostic accuracy of canine scent detection in early- and late-stage lung and breast cancers. Integr Cancer Ther. 2006;5:30-39.
48. Murarka M, Vesley-Gross ZI, Essler JL, et al. Testing ovarian cancer cell lines to train dogs to detect ovarian cancer from blood plasma: A pilot study. J Vet Behav. 2019;32:42-48.
49. Pavlou AK, Magan N, Sharp D, Brown J, Barr H, Turner AP. An intelligent rapid odour recognition model in discrimination of Helicobacter pylori and other gastroesophageal isolates in vitro. Biosens Bioelectron. 2000;15(7-8):333-342
50. Pickel D, Manucy GP, Walker DB, Hall SB, Walker JC. Evidence for canine olfactory detection of melanoma. Appl Anim Behav Sci. 2004;89:107-116
51. Powell NA, Ruffell A, Arnott G. The Untrained Response of Pet Dogs to Human Epileptic Seizures. Animals (Basel). 2021 Jul 31;11(8):2267.
52. Reeve C, Cummings E, McLaughlin E, Smith S, Gadbois S. An Idiographic Investigation of Diabetic Alert Dogs' Ability to Learn From a Small Sample of Breath Samples From People With Type 1 Diabetes. *Can J Diabetes*. 2020;44(1):37-43.
53. Schoon GAA, De Jonge D, Hilverink P. How dogs learn to detect colon cancer—Optimizing the use of training aids. J Vet Behav. 2020;35:38-44
54. Seo IS, Lee HG, Koo B, et al. Cross detection for odor of metabolic waste between breast and colorectal cancer using canine olfaction. PLoS One. 2018;13:e0192629

55. Shelby, R.A.; Schrader, K.K.; Tucker, A.; Klesius, P.H.; Myers, L.J. Detection of catfish off-flavour compounds by trained dogs Aquac. Res. 2004, 35, 888–892.
56. Smith, D.A.; Ralls, K.; Hurt, A.; Adams, B.; Parker, M.; Davenport, B.; Smith, M.C.; Maldonado, J. Detection and accuracy rates of dogs trained to find scats of San Joaquin kit foxes (Vulpes macrotis mutica). Anim. Conserv. 2003, 6, 339–346.
57. Sonoda H, Kohnoe S, Yamazato T, Satoh Y, Morizono G, Shikata K, et al. Colorectal cancer screening with odour material by canine scent detection. Gut (2011) 60:814–9
58. Tacher, S.; Quignon, P.; Rimbault, M.; Dreano, S.; André, C.; Galibert, F. Olfactory Receptor Sequence Polymorphism within and -*Between Breeds of Dogs. J. Hered. 2005, 96, 812–816. [CrossRef] [PubMed]
59. ten Hagen NA, Twele F, Meller S, Jendrny P, Schulz C, von Köckritz-Blickwede M, et al. Discrimination of SARS-CoV-2 infections from other viral respiratory infections by scent detection dogs. Front Med. (2021) 8:588. 10.3389/fmed.2021.749588
60. Thuleau A, Gilbert C, Bauër P, et al. A new transcutaneous method for breast cancer detection with dogs. Oncology. 2019;96:110-113.
61. Walczak, Marta, et al. "Impact of individual training parameters and manner of taking breath odor samples on the reliability of canines as cancer screeners." Journal of veterinary behavior 7.5 (2012): 283-294.
62. Wasser, S.K.; Davenport, B.; Ramage, E.R.; E Hunt, K.; Parker, M.; Clarke, C.; Stenhouse, G. Scat detection dogs in wildlife research and management: Application to grizzly and black bears in the Yellowhead Ecosystem, Alberta, Canada. Can. J. Zool. 2004, 82, 475–492
63. Wasser, S.K.; Davenport, B.; Ramage, E.R.; E Hunt, K.; Parker, M.; Clarke, C.; Stenhouse, G. Scat detection dogs in wildlife research and management: Application to grizzly and black bears in the Yellowhead Ecosystem, Alberta, Canada. Can. J. Zool 2004, 82, 475–492. [CrossRef]
64. Wei, Q.; Zhang, H.; Ma, S.; Guo, D. Sex- and age-related differences in c-fos expression in dog olfactory bulbs. Acta Zool. 2017, 98, 370–376.
65. Williams H, Pembroke A. Sniffer dogs in the melanoma clinic?. Lancet. 1989, 333,734

66. Willis CM, Britton LE, Harris R, Wallace J, Guest CM. Volatile organic compounds as biomarkers of bladder cancer: sensitivity and specificity using trained sniffer dogs. Cancer Biomark. 2011;8:145-153
67. Willis CM, Britton LE, Swindells MA, et al. Invasive melanoma in vivo can be distinguished from basal cell carcinoma, benign naevi and healthy skin by canine olfaction: a proof-of-principle study of differential volatile organic compound emission. Br J Dermatol. 2016;175:1020-1029
68. Willis CM, Church SM, Guest CM, Cook WA, McCarthy N, Bransbury AJ, Church MR, Church JC. Olfactory detection of human bladder cancer by dogs: proof of principle study. BMJ. 2004 Sep 25;329(7468):712
69. Yamamoto A, Kamoi S, Kurose K, et al. The trained sniffer dog could accurately detect the urine samples from the patients with cervical cancer, and even cervical intraepithelial neoplasia grade 3: a pilot study. Cancers. 2020;12:3291

Thesis progress report for veterinary students

Name of student: Avigail Sala Simon

Neptun code of the student:

Name and title of the supervisor: Dr. Zöldág László prof. emer.

Department: Animal Breeding and Genetics

Thesis title: Medical Detection Dogs. Évkihagyás alatt végzi (2023/24).

Consultation – 1st semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2023	febr.	8	Discussion about the chosen thesis topic in general	
2.	2023	march	9	Rough content and design of the planned thesis	
3.	2023	april	18	Improvement of the planned thesis content	
4.	2023	may	5	Literature selection for the thesis	
5.	2023	june	8	Analysis and evaluation of the selected references	

Grade achieved at the end of the first semester: very good (5)

Consultation – 2nd semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2023	sept.	16	Discussion of the prepared rough manuscript	
2.	2023	oct.	20	Final talking over of the ready made manuscript	
3.	2023	nov.	5	Final glance onto the manuscript and approval	

4.	2023	nov	17	Upload	
5.	2023				

Grade achieved at the end of the second semester: very good (5)

The thesis meets the requirements of the Study and Examination Rules of the University and the Guide to Thesis Writing.

I accept the thesis and found suitable to defence,

....

signature of the supervisor

Signature of the student: ....

Signature of the secretary of the department: ....

Date of handing the thesis in 17 November 2023

Appendix 4.

Supervisor's certification

I hereby confirm that I am familiar with the content of the thesis entitled

„Medical Detection Dogs"

Written by **Avigail Sala Simon**
(Student name)

Which I deem suitable for submission and defence.

Date: Budapest, 2023-11-15

Supervisor's name and signature
Dr. Zöldág László Prof. Emer.

Department

ÁLLATORVOSTUDOMÁNYI EGYETEM
Állattenyésztési és Genetikai Tanszék
1078 Budapest, István u. 2.
1400 Budapest, Pf: 2.