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Abstract:

The olfactory capacity of animals has long been used by humans to help with various activities, e.g., hunting, detecting mines, locating people, and diagnosing diseases. Cancer is among the leading diseases causing death worldwide. Several recent studies have underscored the benefit of using scent to detect cancer, and this paper will review the studies using animals to detect tumor scents. A large variety of animals have been used for this purpose—dogs, rodents, insects, and nematodes—and have shown their capacity to detect cancer, with a success rate close to 90%. Here we discuss these studies, their methodologies, and the animal models used. Finally, we discuss the medical perspectives for cancer diagnosis using odors.

Keywords: Cancer odor, olfactory sensor, odor discrimination, volatile organic compounds, behavioral response.

1. Introduction

Cancer is the second leading cause of death in the world, with an estimated 9.9 million deaths in 2020 [1]. For most cancers, it is widely acknowledged that the early determination of a malignant tumor is crucial for increasing the chance of survival. Indeed, most cancer deaths are linked to a delay in the diagnosis, attributable to the fact that early stage cancers are rarely associated with clear symptoms. Early diagnosis usually increases the effectiveness of therapies by treating the tumor at a stage at which it has not yet caused significant tissue damage or transferred metastases to other organs [2]. Thus, any tool allowing early tumor detection would be particularly valuable in the fight against cancer.

For the last 20 years, a new approach for early cancer detection has been explored that is based on the hypothesis that the presence of a tumor could—for various reasons, e.g., the altered metabolism of cancer cells—result in the production of specific volatile substances (volatile organic compounds [VOCs]) that are detectable by chemical analysis techniques [3]. In fact, many diseases can be detected because of the modifications they induce in body odors [4–7]. Among these diseases are infectious ones such as malaria [8] and tuberculosis [9], and chronic diseases such as mucoviscidose [10], asthma [11], and diabetes [12]. Cancer is no exception: several studies have shown that it was possible to detect differences in VOCs emission between healthy people and cancer patients, especially for lung, breast, and prostate cancers [3].

For many animal species, olfaction plays an essential role in both intra- and interspecific interactions [13–16]. For example, scent is involved in the identification of conspecifics, sex, social rank, or reproductive status [17,18]. There is also some evidence that olfactory capacities are used to recognize congeners who are sick following infections by pathogens [19–24].

Because of their olfactory acuity, animals have been used by humans for various tasks, from hunting to scent detection. The first use of animals for their sense of smell involved dogs in a military context to detect objects such as explosives, mines, and dead bodies during the Second World War [25]. Later, in the 1980s, rodents were used for the same reason. Because of their small size, mice could more easily enter small areas without triggering the explosives [26,27]. The dog's sense of smell is regularly used by police to detect explosives [28,29] and drugs [30], and to search for cadavers [25] as well as at the border for biosecurity [31]. For human diseases, it has been established that dogs, trained or not, can detect the imminence of an epilepsy crisis based on changes in their master’s body odor [32,33]. More recently, dogs have been used to detect COVID-19 [34,35]. In addition to dogs, other animal species have been found that can detect body odors, notably rodents [36], bees [37], or drosophila [38]. Other insects such as wasps have also been trained to detect substances such as cocaine [39].

Recent advances in cancer research have shown that the ability of dogs to detect tumor-associated odors could offer a relevant tool for cancer detection [40–42]. The aim of this paper is to review current knowledge concerning the use of various animals to detect cancerous processes in humans. We examined 49 publications reporting results on this topic published between 1989—the year when the first publication in this field was reported—and 2022. We listed the species capable of detecting cancers and analyzed the different methods to test scent discrimination by detailing the advantages and inconveniences of each species. Finally, we explored the potential interest of each animal group for early detection of cancer in humans.

2. Material and methods

Our exhaustive review was conducted by searching for published articles on PubMed (https://pubmed.ncbi.nlm.nih.gov/), Web of Science (www.webofknowledge.com), and Google Scholar (https://scholar.google.com/) that contained the following keywords: "animal smells" or "animal olfaction", or "animal nose" AND "cancer" or "cancer detection" or "cancer odors" or "cancer scent." The literature included articles published in English from 1989 until 2022, excluding systematic reviews or meta-analyses. For each study, in addition to publication year, we extracted the following information: species, type of cancer, odor source (e.g., exhaled air, urine), whether or not animals were naive before being included in the test, as well as specificity and sensitivity of the test. All graphical representations were performed with the software R version 4.0.3 in Rstudio [43] and required the following packages: ggplot2 [44], GGally[45], and cowplot [46].

3. Results

3.1 History of the use of animals in cancer detection

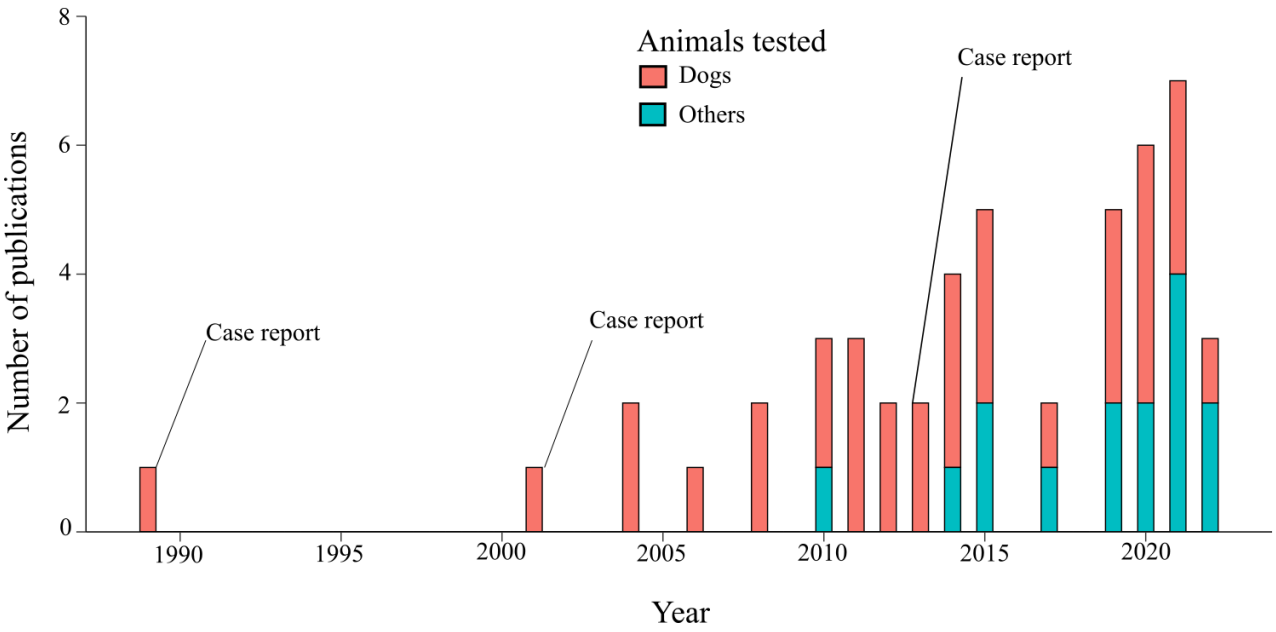


Figure 1: *Number of papers by year published between 1989 and 2022 that addressed the ability of animals to detect cancer odors. Studied animals were divided into dogs and other species, which included nematodes, bees, drosophila, ants, mice, and rats.*

Despite knowledge of the olfactory abilities of animals and their use in different fields, it was not investigated in cancer research until reports of anecdotes from the late 1980s about particular dog behaviors toward owners harboring tumors began to surface. The first study on the subject is a case report in 1989 [47]. This note refers to a 44-year-old woman who presented lesions, which were subsequently diagnosed as malignant melanoma. This patient sought medical care because her two dogs, a Border collie and a Doberman, had detected a lesion by sniffing it. Following this initial observation, no study was launched on the subject. It was not until a second case report in 2001 [48], which described the same scenario, that research was undertaken on the subject. In this second observation of spontaneous identification of cancer by a dog, the owner of a male Labrador retriever was diagnosed with melanoma following his dog's incessant reaction to the lesion (licking and sniffing). Following this second case report, animal studies were undertaken. In parallel, in the 2000s, research showed that it was possible to detect differences in odor profiles between healthy and cancerous individuals [3].

Since these first case reports, 46 studies have been conducted between 2004 and the present on the ability of animals to detect cancer **(Fig. 1; Table S1 and S2)**. Most of these publications (31) concerned dogs, following the above described case studies. Because dogs are already common subjects of research based on sense of smell, many training protocols were available. Other animal species began to be studied from 2010 onwards, although much less frequently compared to studies with dogs **(Fig. 1 & 2)**.

Among the other species studied are mouse and rat. These species are widely used in medical research, including oncology [49,50]. Using genetically modified murine models, it is possible to induce cancers that are very similar to those occurring in humans (e.g., [51,52] and then explore how tumorigenesis induces modifications in the mouse odor bouquet [53,54]. It is also known that olfaction is one of the most used senses in rodents [55–57]. However, only four publications involved mice between 2010 and 2022 [53,58–60] and only one involved rats [61].

More recently—in 2014— invertebrates began to be used in this research field [62]. The emergence of these new study models is certainly linked to the constraint of experimentation on vertebrate animals, when researchers were encouraged to reduce the use of vertebrates and replace them by other models whenever possible. Olfaction in insects is highly developed and is among the most sensitive of the animal kingdom [63]. There are for instance several examples of insects that demonstrate high sensitivity and selectivity in detecting and discriminating odours (Bromenshenk et al. 2003, Schott et al. 2015, Chamberlain et al. 2012; Frederickx et al. 2011). Nevertheless, only three studies have so far tested the olfactory capacity of insects in the context of cancer. In 2014, there was a first publication on drosophila (*Drosophila melanogaster*) [62]; there was another in 2015 on bees (*Apis melifera*) [64], and one in 2022 on ants (*Formica fusca)* [65].

In 2015, a team in Japan began studying the ability of the nematode *Caenorhabditis elegans* to detect cancer [66–72]. This organism, a study model in sensory neurobiology, offers many advantages as a model system, one of which is its small and transparent body, which allows the imaging of neuronal activity in real time [73]. The Japanese team produced seven publications supporting the hypothesis that nematodes can detect and distinguish odors emitted by scent sources from cancerous and healthy humans.

Figure 2 illustrates the percentage of publications made on each animal species. Many different breeds of dogs were used. Certain breeds were selected that excel in olfaction-based tasks. For instance, dogs such as Labrador retrievers, German shepherds, and Belgian shepherds are often used in scenting tasks in the military field because of their drive and high trainability [74]. However, at least 28 other dog breeds of various sizes have been tested for their ability to detect cancer-related odors. More than half of the publications used medium-sized dogs (30–60 cm); 14 different breeds of these sized were tested, especially Labrador retrievers.

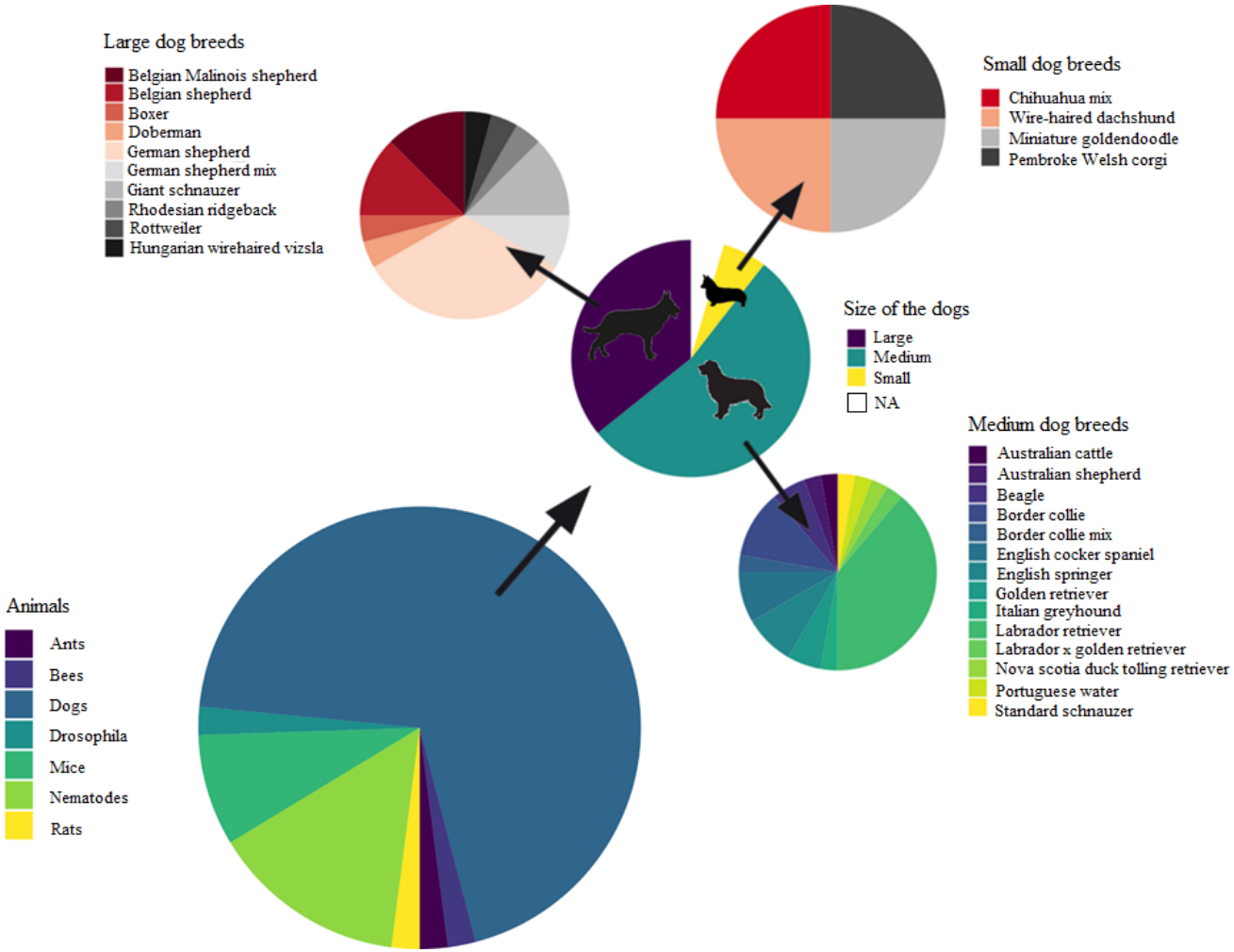


Figure 2: *Proportions of different animals used for odor detection. The dog breeds used (28 different breeds in referenced publications) were classified into three size categories: small, medium, large. Some dogs were crosses between two known breeds, so we were unable to identify the size category to which they belonged and they were therefore classified in the size category "NA."*

3.2 Diversity of cancers

The sense of smell of these different species was used to discriminate different cancers from non-cancer samples. A total of 12 types of cancer were studied, with 10 different odor sources **(Fig. 3)**. Lung cancer was the focus of most studies, with 14 publications, followed by breast cancer (10 publications), ovarian cancer (seven publications), and colorectal, melanoma, and prostate cancers, with five publications each. The greater number of studies concerns lung cancer which is the most prevalent cause of cancer deaths worldwide, and breast cancer which is the most frequently diagnosed cancer in women worldwide [1]. Lung cancer and breast cancer are also the two cancers that have been studied using the largest number of different organisms. Studies on lung cancer have been done mainly with dogs, comprising 75% of the publications, but also mice, rats, and bees. Breast cancer has been tested with dogs, ants, drosophila, and nematodes. Some cancers or stimuli have only been tested using one species, e.g., prostate cancer (five dog studies), cervical cancer (one dog study), lymphoma (one nematode study), leukemia (one nematode study), pancreatic cancer (four nematode studies), and gastrointestinal cancer (three nematode studies). The detection abilities of dogs were tested on a larger number of cancers than for any of the other organisms, eight in all: prostate, ovarian, melanoma, lung, colorectal, cervical, breast, and bladder cancers. Studies using nematodes have included six different cancers (pancreatic, lymphoma, leukemia, gastrointestinal, colorectal, and breast). Among studies conducted using mice, two were on lung cancer [53,60], one on melanoma [75], and one on bladder cancer [58]. Only one publication on lung cancer involved rats [61].

Animals are presented with different sources of stimuli to see if they can discriminate the odors of cancerous individuals. The most commonly used source is urine. Dogs were tested with odors derived from human urine (11 publications) while mice were tested with both human (1 publication) and mouse urine (two publications) and nematodes (one publication with mouse urine and six with human urine). The second most used source is exhaled air from humans, with nine publications involving dogs and one involving rats. Breath sample collection is not invasive to humans—the person only needs to blow into a machine, similar to a stress test by a pulmonologist. Both urine and exhaled breath are simple ways to quickly obtain odor samples. There are other noninvasive methods, such as stool samples or soiled mouse bedding, in which odors emitted by mouse urine, feces, and glands can be found. Sweat is another noninvasive source that has been studied (eg.). These odour sources are not yet widely used, with only two publications using stool samples and one using soiled bedding. These could be further developed, particularly in the context of colorectal and digestive cancers.

~~Surprisingly, the most common odor sources—urine and air—are not the ones that have been tested on most organisms:~~ Cell cultures are often used as odor sources. The three publications involving bees, ants, or drosophila all used odor stimuli from cell cultures. This stimulus was also

tested in three publications with dogs and one with nematodes. The other samples presented to the animals were more invasive and requires either blood sampling or biopsies for tissue or skin samples. Dogs were tested with a wide spectrum of samples, with eight different stimuli used. Nematodes were also tested for different cancers and different stimuli, although most studies were made using human urine [66,67,69–72].

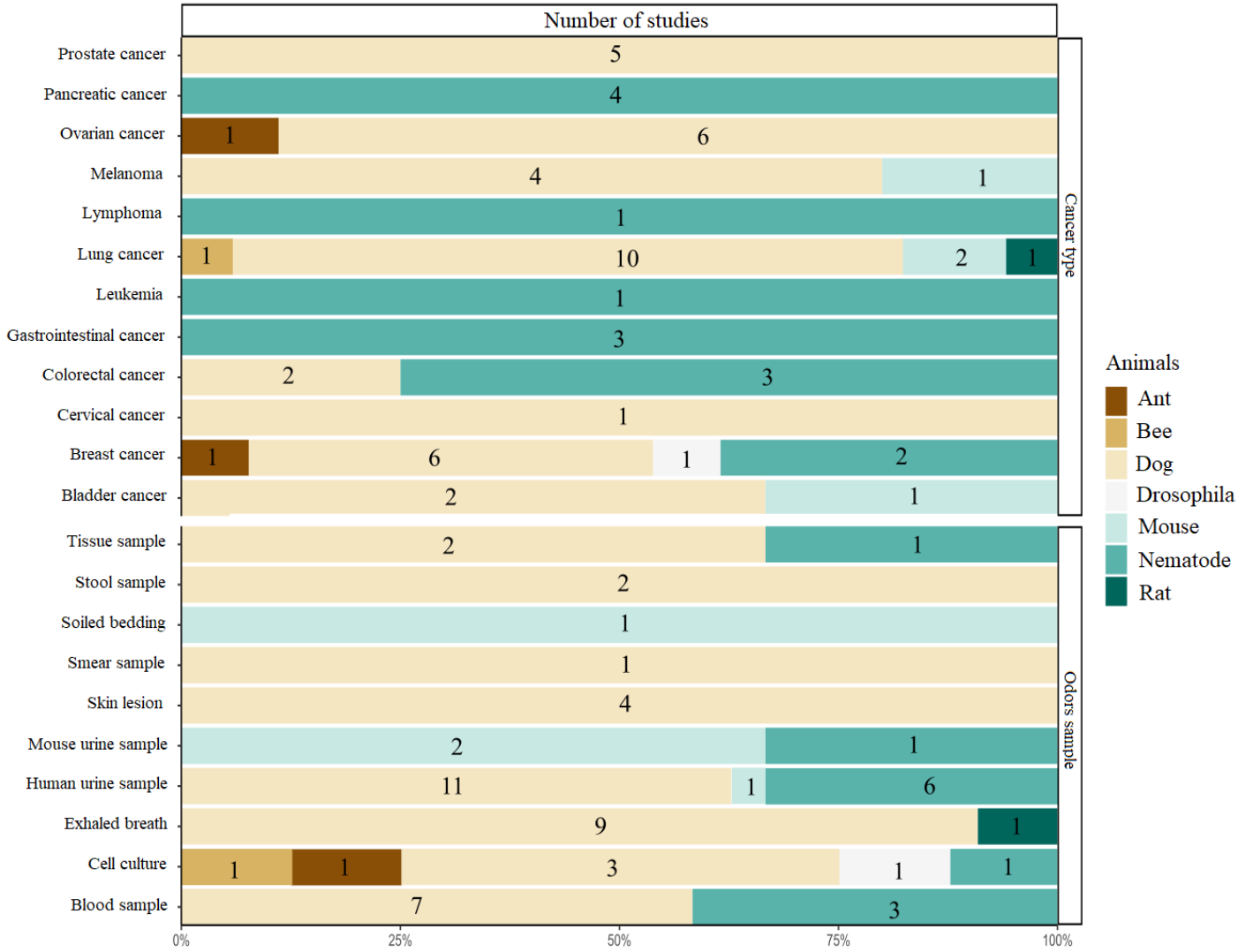


***Figure 3**: Summary of cancers and odor sources used with each animal, including the number of publications identified per cancer type and source of odour.*

3.3 Experimental setup

Animals tested for cancer odor discrimination have different olfactory abilities and behavioral responses, which necessitates adapting the experimental set-up to the chosen animal model. Some methods are exclusive to one organism while others have been be generalized and used on several organisms by adapting the method to the study model. Three main techniques were identified: tests involving conditioning training, behavioral tests carried out without conditioning, and electrophysiology tests **(Table 1 and Experimental setup)**. These tests showed similar overall capacities of the animals, although the tests using bees were inconclusive.

Table 1: *Summary of the advantages and disadvantages of techniques using each species to detect cancer associated odours. Test details are given in the supplementary material.*

Test technique	Species	Advantages	Disadvantages
Conditioning training	Dogs	• Good sensitivity and specificity • A single trained individual is useful for several years • Many samples tested • Animal not killed	• Long training time required (several months). • Cost (over US$50,000 per dog) • Few individuals/replicates • Ethical documents are needed for the experiment
	Bees	• Good sensitivity and specificity • Short testing time (than 3 hours) • The individual can be killed or returned to the colony after the test (even though in the study mentioned bees have been killed)	• No discrimination of cancerous individuals
	Ants	• Good sensitivity and specificity • No ethical documents needed for experimentation • Large number of individuals available • Short testing time (a few minutes) • The individual can be killed or returned to the colony after the test	• Many individuals are necessary
	Rats	• Good sensitivity and specificity • Short training time (a few days) • Animals can be used several times on multiple samples (even though rats in the study mentioned have been euthanized after the experiment)	• Many individuals are necessary • Ethical documents are needed for the experiment
	Mice	• Good sensitivity and specificity • Short training time (a few days) • Animals can be used several times on multiple samples (even though mice in the study mentioned have been killed after the experiment)	• Many individuals are necessary • Ethical documents are needed for the experiment

Behavior test: habituation/ generalization	Mice	• Good sensitivity and specificity • Short test time (a few minutes) • Animals can be used several times on multiple samples (even though mice in the study mentioned have been killed after the experiment)	• Many individuals are necessary • Ethical documents are needed for the experiment • Limited number of tests per individuals
Behavior test: preference	Mice		• No preference for cancerous or healthy stimuli
	Nematodes	• Preference for cancerous stimuli • Good sensitivity and specificity • No ethical documents needed for experimentation • Large numbers of individuals are available • Reduced total duration of the experiment (a few minutes) • No training	• Many individuals are needed for one test • Killed after tests
Electrophysiology test (calcium imaging)	Drosophila	• Good sensitivity and specificity • No ethical documents needed for experimentation • Large number of individuals are available • Reduced total duration of the experiment (a few minutes) • No training	• Many individuals are needed for one test • Killed after tests

Conditioning tests involves learning by a system of reward or punishment **(see Experimental setup)**. This type of test can be used with dogs, insects, and rodents. To estimate the success rate of conditioning tests, we can calculate the sensitivity and specificity as follows:

Sensitivity = CP/ (CP+ MI)

Specificity= CN/ (CN+FA)

where CP indicates a correct positive response, when animals detected the target sample; MI indicates a false negative response, when animals did not detect the target sample; CN indicates a correct

negative response, when animals did not detect a control sample; and FA indicates a false positive alert, when animals detected a blank or control sample.

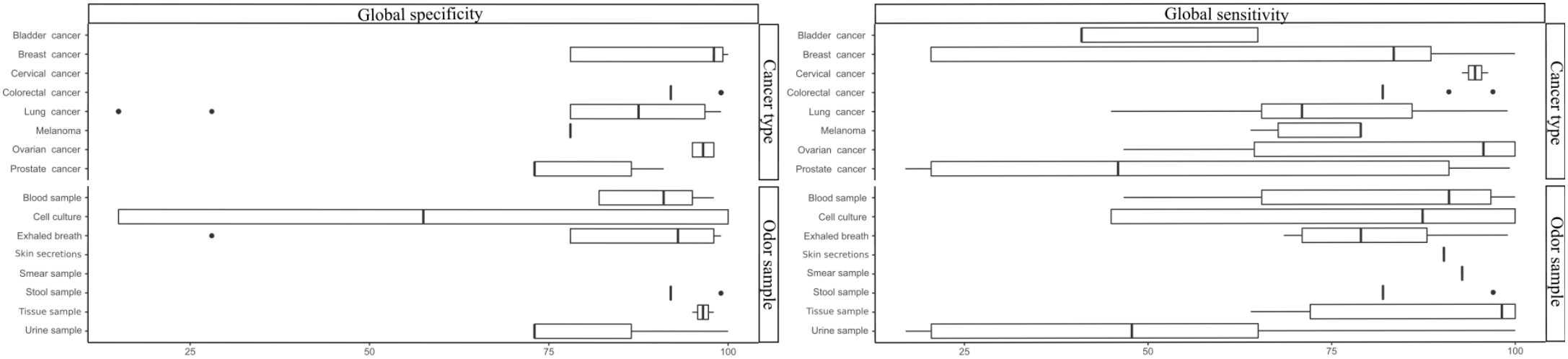
All publications on dogs on average display good sensitivity, i.e., 71%±24, as well as a good specificity, i.e., 85%±18. Some studies obtained scores higher than 90% or even 100% success. For example, using blood serum, beagles had a sensitivity of 96.7% and a specificity of 97.5% [76]. Other studies obtained much less conclusive results, with success rates below 50% [77–79]. Sensitivity and specificity vary among studies, and we noted that they vary depending on the cancers tested and the stimuli presented (Fig. 4). Sensitivity varies more than specificity, which means that dogs are more likely to make false positives - indicating that a sick person is healthy - than false negatives, which can have detrimental consequences for patients in the absence of other diagnostic methods. Detection of urine showed the lowest sensitivity among the stimuli, yet it is the most common stimulus tested on the dogs. Regarding the types of cancers tested, prostate and bladder cancers had average sensitivities close to 50%, making them the least well detected cancers by dogs. However, this poor detection level may be because only urine samples were used and that dogs are less sensitive to cancer related molecules present in the urine.

Of the other animals tested by conditioning, mice showed success rates ranging between 80 and 100% (ref). In another study, mice could distinguish cancerous conspecifics based on their urine. Compared with dogs, either mice are more sensitive to informative molecules present in the urine and/or it might be easier to distinguish diseased conspecifics than non-conspecifics. The rat study used exhaled breath samples to detect lung cancer, resulting in a sensitivity of 82% and a specificity of 81%. The two studies conducted on insects using conditioning tests gave mixed results. Bees did not seem to be capable of discrimination between healthy and cancerous cell cultures with proboscis extension response (PER) tests [64]. The failure of tests involving bees may be explained by the fact that cell cultures do not produce compounds to which the bees are sensitive. While, test based on bees olfaction were successful in other contexts, such as landmine detection [37] or in an ecological context with the use of bees to detect the presence of larvae of the Mediterranean fruit fly [80]. The study using ants [65] showed that when ants were conditioned with sugar water to the odor (healthy cell cultures or cancerous cell cultures), they spent much more time near the conditioned smell than the new smell. These results show that ants are able to distinguish between healthy and cancerous cells and even between two different cancerous cells. Conditioning has advantages and disadvantages, depending on the animal tested. In the context f cancer detection, for all species tested other than bees, this method resulted in sensitivity and specificity above 80%.

Conditioning tests require a learning period that can be variable in length. This training time can be a disadvantage with dogs. Dogs start training in average at 10±4.8 months, with training durations varying greatly among studies (average 41±34 weeks). Some dogs were trained for only 3–4

262 weeks prior to testing [76,81] and others were trained for seven to 24 months [82–84]. This makes the
263 dog the organism that takes the longest to provide conclusive results. For all other species tested, the
264 learning time was much shorter, from a few minutes to a few days.

Figure 4: *Global sensitivity and specificity for all experiments with dogs as a function of cancer type and odor sample. Boxplots represent the first, median, and third quartiles on the 95% extent. The dots represent the outliers and when there was only one data point.*



However, once a dog is trained, its training can last for its lifetime and thus the same individual can be used for a large number of tests, unlike, for example, insects or nematodes, which usually cannot be used more than once, due to their short lifespan. The possibility of using trained dogs throughout their lives makes them an attractive study model. Alternatively, studies involving animals that cannot be used repeatedly require a sufficient number of individuals to be statistically reliable. For example, habituation–discrimination tests typically involve between 10 and 20 mice/rats and 30 or so ants for a given experiment in contrast to dog conditioning studies, where only one to 10 dogs were involved. This results in a large number of organisms to be tested; in the case of rodents, this involves ethical constraints, while experimentation on invertebrates have fewer restrictions.

These advantages and disadvantages including the number of individuals required and the ethical issues are common to all three testing methods—the other two being behavioral tests without prior learning and electrophysiology tests. Behavioral tests were carried out on mice and nematodes. Two types of tests were performed on mice. The first was a habituation/generalization test [60], where the mouse was habituated for 14 minutes to a scent stimulus (cancerous or healthy) and then presented with two new stimuli. The mouse will typically spend more time smelling the stimulus with which it is less familiar. In this way, it can be determined whether mice can differentiate between the smells of cancerous and healthy stimuli. This study showed that mice were able to discriminate between cancerous conspecifics but—as with ants—this depended on the stimulus to which they had been habituated. Mice were able to differentiate a cancerous stimulus from a non-cancerous one if they were habituated to a non-cancerous stimulus, even at an early stage of cancer development. But when a mouse was habituated to a cancerous stimulus, mice showed difficulty discriminating among two different cancerous stimuli. This may be due to possible higher heterogeneity in odors emitted by two cancerous stimuli [85].

The second behavioral test consists of a preference test where two different odors are placed either in a Y-maze for mice or in two Petri dishes for nematodes. Despite their ability to detect the odors, wild-derived mice did not show a preference for healthy or cancerous samples [60]. This was not the case for nematodes, which were attracted to the odors emitted by stimuli of cancerous patients, regardless of the cancer type or stage [66,70,71].

Behavioral tests involving mice or nematodes have good sensitivity and specificity scores. This type of test allows a reduction in testing time by eliminating the need to learn by positive or negative reinforcement. Nevertheless, as mentioned above, the number of individuals required is much larger compared to conditioning with dogs. This disadvantage is not an issue for nematodes since there are no ethical requirements for experimentation, which allows an unlimited number of individuals that could be tested.

The last technique involves electrophysiology. As of now, it has only been tested on *Drosophila melanogaster*, with calcium imaging [62]. For this method, the response of drosophila to olfactory stimuli presented to the fly's antennae, was examined at the brain level using calcium imaging . Even if this study did not specifically identify candidate VOCs, it showed that drosophila has a network of natural chemosensors that seem to respond to odors of medical interest (i.e. [62].

3.4 Medical perspectives

Cancer detection by animals—from dogs or mice to insects and nematodes—seems to be a promising avenue. Indeed, they seem to be able to detect cancer even with only slight variations in body VOCs. This capacity to detect cancer could allow a much more accurate diagnosis than current cancer screening tests, such as MRI. However, there are different experimental setups depending on which animal is used and different behavioral responses and measures, such as digging, rolling over, sitting, rearing, and barking with dogs; physiological measurements such as electroantenography (EAG) coupled with calcium imaging with insects; and attraction or repulsion responses with mice or nematodes. Furthermore, a number of different stimuli can be presented to animals. The optimization of these stimuli for each type of cancer and for each animal must be considered. Some stimuli are currently little used even though they are potentially reliable. Other stimuli, such as urine, are often used even though they do not necessarily give the best results (e.g. in studies with dogs). Furthermore, given the detection capacity of insects, it is important to test stimuli other than cell cultures to see if this capacity could be extended to other commonly used stimuli, e.g., urine or exhaled air.

Twelve different cancers have been studied using animals as cancer detectors. Human cancers (18 in total) have already been studied by chemical analysis of their VOCs to identify molecular differences between cancerous and healthy patients (see the review [3]). However, this review has shown that there was no universal compound associated with cancer odours. It is interesting to note that the cancers most studied by chemical analysis of human VOCs—apart from lung cancer—are different to those that have been investigated by animal detection, e.g., cervical cancer [86].

To make the best use of animals in the field of disease detection and especially for cancer, it is essential to choose an experimental set-up that is easily duplicated and can be extended to a large number of cancers. Based on several studies, it appears that animals can detect cancer at very early stages of precancerous lesions [60,66,83]. This could be useful for initial diagnoses or for cases of relapse, which are sometimes difficult to detect. Early detection is critical to more effectively treat patients. In addition, cancer detection methods must be accurate, fast, simple, and affordable. In this respect, animals that are well trained, or for which a reliable protocol can be established, can help detect early stages of cancer using primary and noninvasive screening. While the different animals that

could be used all have advantages and disadvantages, all offer a non-invasive rapid diagnostic tool for human cancers.

The use of animals could be ethically problematic, especially the use of dogs and mice, so in this context it may seem preferable to use organisms such as insects or nematodes rather than mammalian detectors like dogs for instance. Another alternative is chemical analysis. Techniques such as GC-MS (gas chromatography – mass spectrometry), LC-MS (liquid chromatography coupled with mass spectrometry), and PTRMS (proton transfer reaction mass spectrometry) show promise in the identification of VOCs that can help identify cancerous individuals. Although chemical analysis may be less sensitive than animal "noses" . Further, these analyses are time-consuming and tedious because cancer impact on VOCs does not always involve the same compounds, thus many samples and analyses would be necessary to identify potential cancer biomarkers. Indeed, several candidate VOC molecules have already been identified [3], but there is no consensus on one or more potential biomarkers, although some compounds such as hexanal, acetone, ethanol, or nonanal have been pointed out in several publications [3]. In the absence of general diagnostic markers, the use of animals to detect early stages of cancer development might be more effective.

4. Discussion

The use of animals in the fight against cancer, although effective, is still in its infancy. Indeed, the potential of animals to detect cancer at an early stage has only been explored in the last 20 years. The use of several animals seem promising, with success rates above 80% for most. All species tested have advantages and disadvantages, although from an ethical point of view the development of experiments using invertebrates such as nematodes, ants, or drosophila should be given special attention. Moreover, this ability to detect cancer—with or without learning—opens the door to new questions about how and why animals are able to discriminate these odors.

Many species have evolved highly developed olfactory systems, allowing them to find their prey or food, avoid predators and toxic resources, or to socially interact [13,87]. The need to know one's environment has led animals to develop a highly efficient olfactory system that—with or without training—allows them to identify cancerous individuals. We know about the impact of infectious parasites on the social relationships of several animal species ([20,22,24]), but we know little about cancer. No studies to our knowledge have looked at the ability of conspecifics in the wild to avoid cancerous individuals. Documentation of cancer in natural populations is limited because most wild animals live and die anonymously. The exact prevalence of cancer in a population requires detailed knowledge of the organism under study (e.g., demography, distribution, migration) as well as extensive monitoring and sampling. However, the relative paucity of cancer research in natural

environments does not signify that it is not important in determining the survival of an organism and in shaping ecological dynamics. Indeed, we know that many species—from hydra to humans—can develop cancer [88]. Although there is little information on the prevalence of cancer in nature, some studies have been able to show that cancer can be a significant cause of death in nature [89–92], although population monitoring to investigate the prevalence of cancer is very difficult to set up in the wild. Only Tasmanian devils and beluga have thus far been extensively studied for cancer [93,94].

Concerning the impact of cancer on wild animals, i.e., whether their olfactory capacity allows them to identify a cancerous congener, no study to our knowledge has asked this question. The only study carried out on the impact of cancer on intra- or interspecies relationships was done on tumoral hydras and showed that tumorous polyps were more predated by fish [95]. Is this only due to their larger size, or do they emit different odors that allow fish to differentiate them? For other predators such as the wolf, the dog's ancestor, do they use their sense of smell to detect sick individuals, and can they distinguish cancerous individuals? Are cancerous individuals, which may carry more pathogens following cancer-induced immune suppression [96], excluded from the group or less frequently chosen as sexual partners (e.g., [97])? These questions are even more relevant for species harboring transmissible tumors, such as Tasmanian devils [91]. In this species, the extent to which individuals may avoid each other to prevent contamination is poorly understood. There are many questions about how cancer is perceived in nature by animals and especially how animals perceive cancerous animals using their sense of smell. For species with post-reproductive cancer, selection should not play a role on social interactions since the cancer will not affect the fitness of breeders. However, oncogenic processes may appear at a younger age in some species, or in the case of transmissible cancers, it is possible that selection plays a role in the olfactory capacities of animals to enable them to avoid conspecifics in poor conditions ([98,99]).

Oncogenic agents such as UV radiation, oncogenic contaminants, and hormones released into waterways are increasingly present in the environment due to human encroachment and habitat degradation. Because many of these carcinogens are new, exposed organisms may not have developed effective defense mechanisms and may not have the capacity to avoid them, which might make wildlife particularly vulnerable to carcinogenesis [100–102]. Exploring the extent to which chemical ecology will be central in these novel ecological contexts will be fascinating and will require the integration of concepts and techniques from different disciplines in addition to chemical ecology (e.g., behavioral ecology, physiology, molecular biology, oncology).

5. Concluding remarks

Animals can use their sense of smell to detect objects, drugs, people, or even diseases such as cancer. This ability is shared by many animals that use olfaction as a means of communication in the wild. Their sense of smell allows them to obtain information about the individuals around them, including their age, sex, and health status. Using their ability to identify individuals with parasitic, chronic, or genetic diseases such as cancer is a promising avenue for the early detection of many diseases.

Different experimental techniques could be used with different animals. This review covered olfactory studies that used dogs as well as other species and highlighted the advantages and disadvantages of each species. Analyzing the neural electrophysiological responses (e.g., electroantenography) of species capable of detecting cancer and thus identifying the compounds that are different between cancer patients and healthy ones would be invaluable. However, this involves a vast number of individuals who are destined to be euthanized. In this respect, insects or other invertebrates may be an interesting avenue of research: they have highly developed olfactory capacities and their use in experimentation is not at this time subjected to any ethical constraints. Finally, the ability of animals to detect cancer raises many interesting ecological and evolutionary questions.

Author's Contribution

FG and AP collected the data. FG analyzed the data and wrote the paper. LD, FT, and GG supervised the project and contributed to the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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SUPPLEMENTARY MATERIAL:

Table S1: *List of studies that examined the ability of dogs to discriminate cancer odors, including details on dog breed, sex, age, training duration, previous experience, cancer type, sample type, and the main results of each study.*

Study type	Authors	Year of publication	Cancer type	Number of dogs tested	Breed	Sex	Age	Length of time trained	Previous experience	Odor samples	Sensitivity for each dog	Specificity for each dog	Global detection sensitivity (%)	Global detection specificity (%)
Case reports	[47]	1989	Melanoma	1	Border collie and Doberman	NA	NA		Naive	Skin lesion				
	[48]	2001	Melanoma	1	Labrador retriever	Male	NA		Naive	Skin lesion				
	[103]	2013	Melanoma	1	Alsatian	Female	NA		Naive	Skin lesion				
Prospective study	[104]	2004	Melanoma	2	Standard schnauzer	Male	4 years		Different distinctions. Served 2.5 years in police; certified bomb-detection dog; served in research efforts by the US Department of Defense	Tissue	NA	NA	86%	
					Golden retriever	Female	6 years		Different distinctions				75%	
	[77]	2004	Bladder cancer	6	Mixed breed	Male	6 years	7 months	Obedience	Urine	11%	NA	41%	NA
					Labrador retrievers	Female	7 years				33%			
					Cocker spaniel	Male	1.5 years				55.50%			
						Female	2 years				55.50%			

					Female	5 years				44%			
					Female	7 years				44%			
	[81]	2006	Breast cancer and lung cancer	5	Labrador retriever	Male	7–18 months	2–3 weeks	Recruited dogs were screened for their level of eagerness to sniff objects and respond to commands	Exhaled breath	NA	NA	Lung cancer: 99% Breast cancer: 88%	Lung cancer: 99% Breast cancer: 98%
					Labrador retriever	Male								
					Labrador retriever	Female								
					Portuguese water dog	Male								
					Portuguese water dog	Female								
	[78]	2008	Breast cancer	6	Aussie cocker mix	Male	2 years	12–14 months	NA	Urine	28%	NA	20.5%	NA
					Collie mix	Females	8 years		NA		28%			
					German shepherd		4 years		Tracking, agility, obedience		22%			
					Rhodesian ridgeback		5 years		Agility, obedience, rally		28%			
					Boxer		6.5 years		Agility, obedience, rally		17%			
					Italian greyhound	Male	5 years		Agility		11%			
			Prostate cancer	4	Chihuahua mix	Females	4 years		Service: therapy		6%			
					Miniature goldendoodle		2.5 years		Service: therapy		15%			
					Pembroke Welsh corgi		6 years		Therapy: obedience		22%			
					Border collie		2 years		NA		28%			
	[105]	2008	Ovarian cancer	1	Giant schnauzer	NA	4 years	12 months		Human sample tumor bank	100%	100%	100%	97.5%
	[106]	2010	Ovarian	2	Giant	Females	7 years	9 months	Trained to	Blood and			Blood:	Blood: 98%

			cancer		schnauzer				detect ovarian carcinoma	tissue test			100%	
					Giant schnauzer		3 years	9 months	Naive				Tissue: 100%	Tissue: 95%
	[107]	2011	Prostate cancer	1	Belgian Malinois shepherd	NA	NA	16 months	Chosen among young dogs destined for explosives detection training. No previous training	Urine and blood	91%			
	[108]	2012	Lung cancer	4	German shepherd	2 Males and 2 Females	2.5–3 years	NA	Family dogs	Exhaled breath	NA	NA	71%	93%
					German shepher									
					Labrador retriever									
					Australian shepher									
	[82]	2011	Colorectal cancer	1	Labrador retriever	Female	8 years	6 months (November 2008 through June 2009)	Trained for water rescue beginning in 2003 and then began training as a cancer detection dog in 2005	Human watery stool	91%	99%	91%	99%
										Exhaled breath	97%	99%	97%	99%

	[109]	2010	Bladder cancer	4	Cocker spaniel	Males	6 and 8 years	NA	Obedience commands	Urine	NA	NA	57–73%	56-92%
					Springer spaniel	Male	5 years							
					Labrador retriever	Female	6 years							
	[110]	2012	Lung cancer	NA	German shepherd mix	Male	20–22 months		NA	Exhaled breath	NA	NA	82.2%	82.4%
	[111]	2012	Breast and lung cancer, melanoma	6	German shepherd	Males	3 dogs ≥ 20–22 months; 3 dogs ≤ 6 months	NA	Naive	Exhaled breath	NA	NA	79%	78%
					Labrador retriever									
	[83]	2013	Ovarian cancer	2	Giant schnauzer	NA	10 years	2 years		Blood	Series I: 97%; Series II (3 months after chemo): 50%; 6 months after chemo: 60%	Series I: 99%; Series II (3 months after chemo): 96%; 6 months after chemo: 90%	Serie I: 97%; Serie II: 70% and 80%	Serie I: 99%; Serie II: 95% and 92%
					Giant schnauzer	NA	6 years				Series I: 97%; Series II (3 months after	Series I: 100% Series II (3 months after chemo):		

										chemo): 80%; 6 months after chemo: 100%	94%; 6 months after chemo: 94%		
	[112]	2014	Lung cancer	4	Belgian shepherd	Male	8 years		Scent-tracking missing people, environmental pollutants	Exhaled breath, Urine	Exhaled breath: 76%; urine: 66%	Exhaled breath: 33.3%; urine: 41.7%	66–76%	33-41.7%
					Border collie	Male	4 years		Scent tracking, environmental pollutants					
					Wire-haired dachshund	Male	8 years		Scent tracking					
					Rottweiler	Female	3 years		Scent tracking					
	[79]	2014	Prostate cancer	2	Labrador	NA	9 years			Urine	13%	71%	17%	73%
					Border collie	NA	3 years				25%	75%		
	[113]	2014	Lung cancer	2	German shepherd mix	Male	NA	NA	NA	Exhaled breath	NA	NA	86%	28%
	[114]	2015	Breast cancer	2	Belgian shepherd, Groenendael type	Female	5 years	6 months	Detect explosives and identify people who need to be singled out in a group	Cell culture	NA	NA	100%	100%
					Belgian shepherd, Malinois type	Female	2.5 years							
	[115]	2015	Prostate cancer	2	German shepherd	Females	2–3 years	4 months	Explosives detection	Urine	Dog 1: 100% Dog 2: 98.6%	Dog 1: 98.7% Dog 2: 97.6%	98.7–100%	97.6-98.7%
	[64]	2015	Lung cancer	2	Labrador retriever	Female	3 years	8 months	Both dogs had basic obedience training and were experienced in scent	2 lines of cell culture	40%	50%	45%	15%
					Mixed breed	Male					20%	10%		

								discrimination					
[86]	2017	Cervical cancer	1	Beagle	Male	3 years		Trained for drug detection	Human fresh biopsies; smear sample	Smears: 92.78%; Surgical bandages: 96.36%	Smears: 99.1%; Surgical bandages: 99.55%	Smears: 92.78; Surgical bandages: 96.36	Smears: 99.1; Surgical bandages: 99.55
[76]	2019	Lung cancer	3	Beagle 1	NA	2 years	4 weeks	Naive	Blood serum	91%	100%	96.7%	97.5%
				Beagle 2						100%	100%		
				Beagle 3						82%	97%		
[116]	2019	Ovarian cancer	1(4 started training)	German shepherd	Female	33 months	NA	Naive	Cell culture	87.40%			
				German shepherd	Female	12 months							
				Labrador retriever	Male	49 months							
				German shepherd	Female	50 months							
[117]	2019	Breast cancer	2	Belgian shepherd	Males	18 months	5 months	Naive	Skin secretions	100%	100%	90.3%	90.3%
				Belgian shepherd						88.9%	88.9%		
[118]	2020	Ovarian cancer	4	German shepherd	3 Females / 1 Male	2–7 years	NA	NA	Blood plasma	False alarm: 14–41%; hesitated: 7-42%	NA	24–56%	NA
				Labrador retriever									
				English springer spaniel									
[84]	2020	Lung cancer	3	Belgian Malinois	Female	5 years	1 year	NA	Urine	First trial 45%; second trial 55%	First trial 89%; second trial 91%	54.60%	90.30%
				Belgian Malinois	Female	3 years				First trial 55%; second trial 36%	First trial 91%; second trial 87%		

					Mixed breed	Female	3 years				first trial 73%; second trial 64%	First trial 91%; second trial 93%		
	[119]	2020	Ovarian cancer	3	English springer spaniel	NA	12–20 months	6 months	NA	Blood plasma	95.1%	NA	95.70%	NA
					German shepherd						98.7%			
					Labrador retriever, yellow						93.3%			
	[120]	2020	Colorectal cancer	8	Nova Scotia duck tolling retriever	Female	2 years	NA	Privately owned	Stool	74%	94%	82%	92%
					Labrador × golden retriever	Female	2 years		Rejected as guide dog		88%	91%		
					English cocker spaniel	Female	1 years		Working dog line		88%	87%		
					English cocker spaniel	Female	1 years		Working dog line		79%	90%		
					Labrador × golden retriever	Female	2 years		Rejected as guide dog		83%	90%		
					Labrador × golden retriever	Female	1 years		Rejected as guide dog		80%	91%		
					English cocker spaniel	Male	1 years		Working dog line		80%	95%		
					Belgian Malinois	Female			Working dog line		84%	96%		
	[121]	2021	Prostate cancer	2	Labrador retriever	Female	4 years	3 years	NA	Urine	71.4%	76.2%	71.4%	73%
					Wire-haired Hungarian Vizsla	Female	7 years				71.4%	70.0%		
	[122]	2021	Breast cancer	1	Labrador retriever	Female	9 years	12 months	Water rescue dog, and then, because of her	Urine	100%	100%		

								high ability of sniffing out and eagerness, she was recruited to have a cancer detection training					
	[123]	2022	Lung cancer	2	Labrador retriever		4 years	21 months	Naive	Blood, Exhaled breath	blood: 69% breath: 62%	blood: 67% breath: 71%		
					Australian cattle dog		5 years	15 months	Naive		blood: 62% breath: 75%	blood: 97% breath: 90%		
	[124]	2021	Lung cancer	1	Golden retriever		7 years	1 year	Raised to discriminate different smells e.g., chamomile tea, cinnamon, or coffee	Exhaled breath	78%			
										Urine	87.80%			

Table S2: *List of studies that examined the ability of rodents and invertebrates to discriminate cancer odors, including details on cancer type, animal type, sex, and age, training type and duration, odor source, and the main results of each study.*

Animal	Authors	Year of publication	Cancer type	Sex	Age (weeks)	Number of animals tested	Training	Training duration	Odor samples	Success or p-value
Mouse	[53]	2010	Lung	Males		6	Y training maze with water recompense	14 days	Mouse urine	Discrimination success > 80%
				Female		1				
	[58]	2017	Bladder	Males	3 weeks	23	Y-maze behavioral assay with water recompense	1–2 days	Human urine	100%
	[75]	2020	Melanoma	Females	9 weeks	11	Spontaneous preference/avoidance in the open files and Y-maze tests	few days	Mouse urine	Non-significant preference for conspecifics with melanoma
				Males		15				P <0.01 spontaneous from conspecifics with melanoma
				Females		20	Trained mice tested in Y maze with treats as a recompense			P > 0.001; mice discriminated cancerous urine with 90% probability of choosing melanoma samples and 82% for samples from mice with or without a visible melanoma tumor
				Males		20				
	[60]	2022	Lung	Females	12 weeks	18	Spontaneous preference/avoidance in Y-maze tests	10 min test	Soiled mouse bedding	Non-significant preference for conspecifics with melanoma
				Males	12 weeks	11–16	Habituation/generalization tests	21 min test	Soiled mouse bedding	p<0.01; mice discriminate cancerous mice at early and late stages of cancer development

Rats	[61]	2021	Lung	Males	4 weeks	12	Trained rats tested to discrimination reinforcement / reward / punishment	30 min once a day	Exhaled breath	Sensitivity: 82%; Specificity: 81%
Drosophila melanogaster	[62]	2014	Breast	Females	1–5 days old	NA	Calcium imagery	A few minutes	Cell culture	Significant difference
Honeybee (*Apis mellifera*)	[64]	2015	Lung	Females	NA	20 in total, 14 tested	Test of proboscis extension (PER)	1 h	Cell culture	No discrimination (p = 0.062)
Ant *(Formica fusca*)	[65]	2022	Ovarian and breast	Females (foragers)	A few days	36	Conditioning test with reward sugar solution + memory tests	A few minutes	Cell culture	Discrimination between healthy and cancerous, but not between two cell culture lines
Nematode (*Caenorhabditis elegans*)	[66]	2015	Colorectal, gastric, breast		Young adults	50–100	Preference tests in Petri dish with one stimulus (healthy or cancerous) on each side	A few minutes	Cell culture, tissue, blood, urine	Sensitivity: 95.8%; Specificity: 95.00%
	[67]	2019	Colorectal, gastric			50–100			Urine	61.1–82.1%
	[68]	2019	Pancreatic			50			Mouse urine	Sensitivity: 71.40%; Specificity: 83.30%

	[69]	2020	Gastrointestinal			50			Urine, blood	80–90%
	[72]	2021	Pancreatic			50–100			Urine	Sensitivity: 84.6%; Specificity: 60%
	[71]	2021	Breast, esophageal, gastric, colon, gallbladder, bile duct, pancreatic, malignant lymphoma, acute myeloid leukemia			100			Urine	Sensitivity: 87.50%; Specificity: 90.20%
	[70]	2021	Pancreatic cancer			50–100			Blood, urine	P < 0.001

903

Experimental setups:

I) Conditioning training

Two types of conditioning training can be used in animals: classical conditioning and operant conditioning [125].

- **Classical conditioning**

Classical conditioning, which is based on the Pavlov reflex, implies that certain stimuli automatically provoke certain responses [126]. The first step in classical conditioning is to associate a neutral stimulus that initially has no effect on the animal—for example, the smell of a cancerous individual—with an unconditioned stimulus, such as sugar, which provokes an unconditioned response. As the neutral stimulus is closely followed by an unconditioned stimulus that naturally triggers an unconditioned response, the neutral stimulus is associated with an unconditioned stimulus, so that the association between the two becomes stronger and the unconditioned response develops. Based on Pavlov's principle, procedures have been adapted to insects that are more commonly called differential conditioning of the proboscis extension response (PER) [127,128].

- **Operant conditioning**

The other type of learning is operant conditioning, which refers to the fact that a behavior can be controlled by its consequence (reward, called reinforcement, or punishment). The animal's behavioral response to a stimulus will be followed by a reinforcement, i.e., the response has a favorable result such as food [129]. In the case of negative reinforcement, the animal will be subjected to an aversive stimulus such as an electric shock, or bitter or unpleasant food [130]. This learning technique is used to train dogs and rats, and for some studies on mice. With conditioning, two parameters are calculated to evaluate detection capacity—sensitivity and specificity.

II) Behavioral tests

- **Habituation/ generalization tests**

The concept of these tests is to habituate an individual to an odor for a given period of time and then present him two new odors; it is assumed that the odor to which the organism will go more is the one that is more different [131].

- **Preference tests / choice tests**

The choice tests take place in a Y-shaped enclosure or another container such as a Petri dish for small organisms. These containers allow the individual to move towards one of the two stimuli presented, which are placed at each end of the olfactometer branch or on opposite sides of the Petri dish. The time spent in each position indicates the preferred stimulus.

III) Electrophysiology test

The last type of test used to determine whether animals can detect cancer consists of analyzing the neuronal response of an insect's antenna to an odorant stimulus. For these tests, insects must be killed before antennae are removed from the animal to perform the experiments.

- **Electroantennography (EAG)**

Electroantennographie (EAG) is a bioassay widely used in experimental entomology for the detection of volatile compounds. This technique consists of analyzing what the insect's antennal olfactory receptor detects. A neurological response recorded from the antenna indicates whether the compound is detected by the organism. For this purpose, the antenna of an insect is connected to two electrodes. The antennae are exposed to an odor (a single compound or mixture of volatile components) and the electric response of the antenna is recorded by the electrodes via an amplifier [132,133]. This technique has never been used to determine whether an insect is able to detect the smell of a cancerous individual.

- **Calcium imaging**

This technique allows an even finer level of detection compared to EAG. It consists in visualizing the activated areas of the olfactory lobes of an insect during olfactory perception [134].