Animal Sentinels for Environmental and Public Health

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ABSTRACT

Studies of the effects of environmental exposures on domestic and wild animals can corroborate or inform epidemiologic studies in humans. Animals may be sensitive indicators of environmental hazards and provide an early warning system for public health intervention, as exemplified by the iconic canary in the coal mine. This article illustrates the application of animal sentinel research to elucidate the effects of exposure to traditional and emerging contaminants on human health. Focusing on environmental issues at the forefront of current public health research, the article describes exposures to community air pollution, environmental tobacco smoke, and pesticides and associations with cancer, reproductive outcomes, and infectious diseases. Finally, it covers the role of marine mammals in monitoring the health of the oceans and humans.

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The concept that animals may serve as sentinels of environmental hazards that have implications for public health is not novel. The familiar image of the canary in the coal mine remains relevant in the 21st century. An article appearing in a 1914 issue of the *Journal of Industrial and Engineering Chemistry* provides a simple description of the concept:

> Birds and mice may be used to detect carbon monoxide, because they are much more sensitive to the poisonous action of the gas than are men. Experiments by the Bureau of Mines show that canaries should be used in preference to mice, sparrows, or pigeons, because canaries are more sensitive to the gas. Rabbits, chickens, guinea pigs, or dogs, although useful for exploration work in mines, should be used only when birds or mice are unobtainable, and then, cautiously, because of their greater resistance to carbon monoxide poisoning. . . . Breathing apparatus must be used where birds show signs of distress, and, for this reason, birds are of great value in enabling rescue parties to use breathing apparatus to best advantage.[1]

Just as miners carried caged canaries during the early part of the 20th century to warn of high levels of carbon monoxide or other impurities in the air, pet, agricultural, and wild animals have been used to assess and predict the effects of environmental contamination in human populations. In a modern example, canaries were used after the sarin release into the Tokyo subway by a terrorist cult in 1995 to detect the gas at the cult's compounds.[2]

Pet animals share the environment and are exposed to many of the same agents as their human companions. Children may be exposed through similar pathways, such as household dust. Animals suffer a similar spectrum of disease as humans and, therefore, may be sensitive indicators of environmental hazards and provide an early warning system for public health intervention. Several historical examples illustrate animals' usefulness as predictors of human illness. In the 1870s, fattened cattle experienced high mortality at a stock show in London's Smithfield market associated with a dense industrial fog—a precursor to the air pollution episodes typified by the infamous London Fog of 1952, during which thousands of residents died.[3] In the 1950s, recognition of neurobehavioral disturbances in the cat population of Minamata, Japan, preceded a severe episode of neurologic disease among local residents caused by consumption of seafood contaminated with methylmercury.[4] Sediments, shellfish, and fish in Minamata Bay became contaminated with mercuric chloride as the result of effluent discharges from a chemical plant. The ataxic "dancing cats of Minamata" were a warning sign. Unfortunately, it was not recognized in time to prevent the human epidemic. In 1962, cases of lead poisoning in cattle and horses living in the vicinity of a smelter alerted the Minnesota state health department to conduct surveillance for lead exposure in the local human populations.[5]

This article addresses the use of animal sentinels as models for epidemiologic studies of human diseases and environmental exposures. Observational studies of spontaneous animal disease in populations can provide additional insights not available from laboratory-based studies of experimental animals.[6] The advantages of using animals as sentinels or comparative models of human disease accrue in part from their relative freedom from concurrent exposures, bias due to confounding, and, to some extent, exposure misclassification. In studies of humans, the influences of cigarette smoking, alcohol, or occupational exposures may obscure an effect of community exposure to environmental hazards. Further, the relatively short latent periods for cancer and other disorders in animals compared with those for humans create an advantage in studying spontaneous diseases in animal models.[7] The accuracy of exposure assessment is a major challenge in environmental epidemiology. The restricted daily mobility and lower frequency of migration over an animal's shorter lifespan contribute to the likelihood that exposure assessment can be conducted more accurately in studies of animal diseases.[7]

Much of the work involving the use of sentinels to identify environmental hazards has focused on cancers in pet animals, particularly dogs, which share the environment intimately with humans.[7,8] Spontaneous canine neoplasms provide useful models for studying the health effects of environmental hazards. Many canine cancers resemble those in humans in biological behavior, pathologic features, proportional morbidity, and recognized risk factors. A classic example of a canine cancer sentinel is the study of mesothelioma by Glickman et al.[9] The authors identified exposure to asbestos through the activities of the owner at work or through a hobby as a significant risk factor accounting for most of the cases. Chrysotile asbestos bodies were identified in lung tissue. There also was a significant association with the use of flea repellants, some of which contained asbestos-like fibers. The findings illustrated the usefulness of epidemiologic research to identify environmental health hazards for humans who share the environment with their pets. Thus, the diagnosis of canine mesothelioma provides an early warning system for the human disease and constitutes a true sentinel event.

This article describes pet and wildlife animal sentinels. These models increase understanding of health effects associated with exposures to environmental hazards relevant to public health.

AIR POLLUTION

An early reference to the use of pet dogs to study lung cancer risk associated with urban air pollution is attributed to V. S. Rosinov, a Russian scientist who claimed that the incidence of lung cancer among dogs in larger cities was higher than that in dogs from rural areas.[10] In the mid-1960s, researchers began exploring the effects of exposure to urban air in Philadelphia. Radiographic screening tools, including modified photo-fluorography originally used for detection of pulmonary tuberculosis, were developed for identification of canine lung cancer and other disorders.[11] Hospital data were used to explore an urban-rural gradient for respiratory tract neoplasia in a case-control study of dogs with primary pulmonary carcinoma, carcinoma of the nasal passages and paranasal sinuses, and carcinoma of the tonsil.[12] The environment was divided into urban and rural segments based on atmospheric pollution data for the city of Philadelphia and the locations of major industries. No elevations in risk for an urban residence were found in the distribution of lung cancer or nasal cancer cases compared with gastrointestinal cancer controls or the total hospital population. However, a significant urban association was noted for carcinoma of the tonsil (odds ratio [OR] = 3.3; 95% confidence interval [CI] 1.6, 6.9). Interestingly, an association between carcinoma of the tonsil and "town-kept" dogs was described in London as early as 1939.[13]

Radiographic techniques were also used to assess the prevalence of non-specific chronic pulmonary disease (CPD) in urban and rural dogs screened at veterinary teaching hospitals in Ithaca, New York, Boston, and Philadelphia.[14] Radiographs obtained during routine clinical workups were graded for evidence of CPD without knowledge of the animal's age or residence, and those with obvious disease process, such as pneumonia, were excluded. In Philadelphia, the prevalence of CPD was significantly higher in older dogs living in the urban area.[14] In an analysis of 1,892 dogs from the three hospitals, the prevalence of CPD was significantly higher in older dogs from the more heavily polluted zones of Boston and Philadelphia compared with dogs from the referent area.[15] These early studies supported the hypothesis that an urban factor, likely related to ambient air pollution, was associated with the development of pulmonary disease in this animal model.

Approximately 30 years later, investigators studied the lungs of dogs from Mexico City and less polluted areas of the country and found structural lung changes, including mononuclear cell infiltrates, smooth muscle hyperplasia, peri-bronchiolar fibrosis, and vascular lesions, that represented an inflammatory response resulting from chronic exposure to particulates and ozone.[16] The pathologic changes described in the lungs of Mexico City dogs were consistent with the radiographic abnormalities identified in Philadelphia dogs and with lung lesions found in dogs from that population.[17] More recently, these investigators studied urban dogs from Mexico City and found histologic evidence of neuroinflammation and an increased abundance of messenger ribonucleic acid from two inflammatory genes in the brains of the dogs. The findings were correlated with decrements in performance on psychometric tests in children similarly exposed to ambient air pollution.[18]

ENVIRONMENTAL TOBACCO SMOKE

The first evidence that exposure to environmental (secondhand) tobacco smoke (ETS) may cause lung cancer was based on a cohort study of Japanese women that showed higher mortality rates among women married to smoking husbands.[19] Since then, multiple studies have confirmed that exposure to ETS is associated with an approximate doubling of lung cancer risk among exposed household residents.[20] The role of household exposure to ETS as a risk factor for cancer in pet dogs was first explored in 1992.[21] Lung cancer cases ($n=51$) and controls with other forms of cancer were obtained from two veterinary teaching hospitals. Exposure assessment included the number of smokers in the household, the amount smoked by each, and the proportion of time spent indoors by the pet. A non-significant relationship was found for exposure to a smoker in the home (OR=1.6; 95% CI 0.6, 3.7) after controlling for confounding. Evidence of a dose-response with number of smokers, packs smoked per day, or an exposure index that included the time the dog spent indoors was not found. However, the dog's skull shape modified the effect: in breeds with short and medium-length noses, the risk increased (OR=2.4; 95% CI 0.7, 7.8). Although results from this small study were inconclusive, it demonstrated the feasibility of using pets to examine the potential effect of household exposures.

A second hospital-based study (103 cases, 378 controls) tested the hypothesis that exposure to ETS increases risk for nasal and sinus cancer—a more common form of cancer in dogs.[22] Exposure to ETS was evaluated by determining the number of smokers in the household, the number of packs smoked per day at home by each smoker, the number of years that each person smoked during the dog's lifetime, and the proportion of time spent indoors by the dog. The adjusted OR for exposure to ETS was 1.1 (95% CI 0.7, 1.8). However, skull shape was again found to exert a strong modifying effect. Among long-nosed dogs, the

OR for a smoker in the house was 2.0 (95% CI 1.0, 4.1). A step-wise increase in the ORs across strata of total packs smoked and total indoor exposure to ETS was found for this group, with a risk of 2.5 (95% CI 1.1, 5.7) for the highest stratum.[22] Urinary cotinine, a metabolite of nicotine, was used as a biomarker to validate questionnaire responses for ETS exposure in 111 dogs and was highly correlated with the exposure metrics used in the case-control studies (Unpublished data, Reif et al., Colorado State University, Department of Environmental and Radiological Health Sciences, 1996). Bertone-Johnson et al.[23] later confirmed the effects of canine household exposure to ETS in a study that found concentrations of urinary cotinine were significantly related to the number of cigarettes smoked by household members and to short nose length in exposed dogs. Exposure to ETS has also been associated with an increased risk of malignant lymphoma in cats,[24] but the findings have not been confirmed in further studies. Collectively, these studies support the hypothesis that exposure to ETS in the home increases risk for respiratory tract cancer in dogs. From a public health perspective, smoking cessation can be recommended as a measure to reduce the incidence of canine, as well as human, respiratory tract disorders and may provide additional motivation for concerned pet owners to quit.

PESTICIDES

Canine malignant lymphoma

Exposures of pet dogs to pesticides have been associated with increased risk for malignant lymphoma and testicular and bladder cancer.[25] Canine malignant lymphoma (CML) is a common cancer of dogs and a model for non-Hodgkin's lymphoma (NHL) in humans. Exposure to pesticides has been suggested as an explanation for the increased risk for hematopoietic cancers such as NHL among farmers in the United States, Sweden, and New Zealand.[26] Several studies have found associations with exposure to phenoxyacid herbicides such as 2,4-dichlorophenoxyacetic acid (2,4-D)—used extensively in agricultural, public, and residential settings to control the growth of broadleaf weeds[27,28]—and with other classes of pesticides.[29] A large, hospital-based case-control study assessed the risk of dogs developing CML from exposure to lawn herbicides.[30] Dogs with CML were 30% more likely to have lived in a home where the owners had applied 2,4-D or employed a commercial lawn care company to treat their yard. The risk rose to a twofold excess when the owners reported four or more herbicide applications yearly; a statistically significant trend was found

for the number of applications. These findings were supported by a biomonitoring study that determined the extent to which dogs absorb and excrete 2,4-D in urine after contact with lawn herbicides.[31] Dogs living in and around residences with recent 2,4-D treatments were shown to absorb and excrete measurable amounts of the herbicide through normal activities and behaviors associated with lawn contact.

Canine testicular cancer

Pesticide exposures have also been associated with an increased risk of testicular cancer in a series of studies of U.S. military working dogs that served in Vietnam with their handlers.[32,33] Increased rates of testicular cancer and testicular dysfunction among dogs that served in Vietnam compared with those that had remained in the United States led investigators to hypothesize that exposures to pesticides including picloram, malathion, and the phenoxyacid components of Agent Orange (2,4-D and 2,4,5-trichlorophenoxyacetic acid) could have been responsible for the selective increase in the incidence of testicular cancer.[32] However, exposures to therapeutic agents used to prevent erlichiosis and malathion used to prevent tick infestation could not be ruled out as contributing factors. In a follow-up study, investigators evaluated the service records of the Vietnam dogs to determine the zones where they were deployed, but were unable to accurately document exposures to chemical defoliants.[33]

Identification of an increased risk for seminoma among military working dogs raised the possibility that this finding could be an indicator of increased risk among soldiers who served in Vietnam. Researchers conducted a case-control study to determine whether Vietnam veterans experienced an increased risk for testicular cancer. While Vietnam service was reported more frequently among cases of testicular cancer than among age-matched controls, the investigators were unable to determine whether the increased risk was attributable to pesticide exposures.[34]

Canine bladder cancer

The risk of human bladder cancer is strongly associated with occupational exposures to chemicals and cigarette smoking. Canine bladder cancer may serve as a sentinel for chemical exposures in general and, more specifically, for exposure to pesticides in the home.[7] An ecologic study of dogs from 13 veterinary teaching hospitals found the proportional morbidity rates for canine bladder cancer to be associated with the county's level of industrial activity. Bladder cancer mortality rates showed a similar correlation with industrial activity in the same counties, suggesting that

a common exposure to environmental carcinogens might be responsible.[35]

Several case-control studies have explored associations between canine bladder cancer and pesticide exposures. A study investigating exposures to ETS, household chemicals, and pesticides found a significant association for the use of topical insecticides in the form of shampoos and dips.[36] The adjusted risk increased with the number of flea and tick dips, and rose to 4.2 (95% CI 1.4, 12.7) for more than two applications per year. The analysis suggested that other compounds found in flea and tick products could contribute to the increased bladder cancer risk. Petroleum distillates and organic solvents including benzene, toluene, and xylene are among the inert ingredients used as carriers and are recognized carcinogens that could represent a hazard for exposed people.[7,36]

Further investigations of the role of pesticides in canine bladder cancer were carried out in Scottish terriers, a high-risk breed with approximately 18 times the risk for bladder cancer compared with mixed-breed dogs.[37] In a case-control study of 83 Scottish terriers with bladder cancer and a similar number of breed-matched controls, the risk of bladder cancer was significantly higher among dogs exposed to lawns or gardens treated with herbicides or insecticides (OR=7.2; 95% CI 2.2, 24.1), including phenoxy herbicides (OR=4.4; 95% CI 1.7, 11.2), but not among dogs exposed to lawns or gardens treated with insecticides alone.[37] The use of high-risk, genetically susceptible breeds permits evaluation of gene-environment interactions and holds promise for elucidation of cancer induction mechanisms in the sentinel animal.

Recently, no association was found between lifetime exposure to chlorinated drinking water and bladder cancer in dogs.[38] Chlorination disinfection byproducts have been suggested as a cause of bladder cancer in humans with long-term exposure.[39]

OCEANS AND HUMAN HEALTH

A robust effort to identify and study animal sentinels of human health in the world's oceans has developed over the past 50 years. The effects of exposures to xenobiotics in the marine environment may be expressed at multiple trophic levels of the ecosystem; however, attention is focused on marine mammals for several reasons.[40] Marine mammals have relatively long lifespans that permit the expression of chronic diseases including cancer, abnormalities in growth and development, and reproductive failure. As apex predators, marine mammals feed at or near the top of the food chain. As the result of biomagnification, the levels of

anthropogenic contaminants found in marine mammal tissues are typically high, often higher than those found in humans. Further, the subdermal blubber layer provides a repository for lipophilic contaminants, particularly organohalogen compounds.[40] Finally, the application of clinical examination procedures and hematological biochemical, immunological, and microbiological techniques, combined with pathological examination, has led to the development of health assessment methods at the individual and population levels.[41,42] With these tools in hand, investigators have begun to unravel the relationships between exposure to environmental chemicals and disease endpoints in marine mammal sentinels.

Organochlorine compounds and heavy metals
The initial steps in studying the effects of environmental pollution on marine mammals were directed at exposure assessment. Investigators worldwide analyzed the levels of heavy metals and organochlorine compounds in blubber and other tissues from dead animals found stranded on beaches or from live-caught animals. As reviewed by O'Shea and Tanabe,[43] the accumulation of data regarding levels of chemical residues in the tissues of marine mammals expanded dramatically during the latter half of the 20th century. By the end of the 1960s, high concentrations of mercury, chlorinated pesticides including dichlorodiphenyltrichloroethane (DDT) and its metabolites, polychlorinated biphenyls (PCBs), and other contaminants had been widely documented in pinnipeds and cetaceans. Multiple studies establishing exposures to marine pollutants were published in the 1980s and 1990s, and attempts at incorporating biomarkers were initiated. This period also saw the first attempt to reproduce adverse effects on reproductive success experimentally by feeding fish containing high concentrations of PCBs to captive harbor seals.[44] A similar effect on reproductive success was reported subsequently in a population of bottlenose dolphins maintained by the U.S. Navy. In this observational study, higher concentrations of DDT metabolites and the sum of 10 PCB congeners were found in blubber of female dolphins whose calves were stillborn or died within the first 12 days of life compared with dolphins whose calves survived for six months or longer.[45]

Effects on the immune system, infectious diseases, and cancer
In the 1990s, evidence began to accumulate that exposure to organochlorines led to a decrease in immunocompetence in highly exposed pinnipeds.[46,47] These findings led to the hypothesis that exposure to organochlorines may be a co-factor in the extensive mortality

experienced by pinnipeds and cetaceans infected with novel strains of morbilliviruses that emerged during the late 1980s and early 1990s.[48,49] The hypothesis has been difficult to confirm since the morbillivirus itself is a potent immunosuppressive agent. Further links between exposure to immunosuppressive agents in the marine environment and infectious diseases were found in a study in which exposure to PCB congeners was compared between harbor porpoises that died from infectious diseases and those that died from physical trauma.[50] A significant incremental increase in risk was found, corresponding to a 2% increase in risk of infectious disease mortality for each 1-milogram/kilogram increase in blubber PCBs.

Exposure to pollutants in the marine environment has also been linked to an increased risk of cancer in exposed marine mammal populations. The observation that beluga whales inhabiting the St. Lawrence estuary (SLE) had an unusually high prevalence of neoplasia compared with the relatively low (rare) prevalence in cetaceans, generally, and in belugas from the Arctic, specifically,[51] led to a series of studies to address the hypothesis. SLE beluga whales live in a heavily polluted environment that receives discharges from the industries, such as aluminum smelters, along the coastline of the estuary.[52] Adducts to benzo[a]pyrene, a carcinogenic polycyclic aromatic hydrocarbon, were demonstrated in SLE belugas, but not in belugas from the Arctic, and high concentrations of PCBs were found in tissues of SLE belugas.[53] A heterogeneous group of tumors that included intestinal and gastric adenocarcinomas, mammary gland adenocarcinomas, ovarian granulosa cell tumors, lymphosarcoma, and bladder carcinoma was reported, consistent with the hypothesis that exposure to polycyclic aromatic hydrocarbons may be involved in the etiology of cancer in these animals. If the hypothesis is correct, the findings have important implications for contiguous human populations.[52]

A second cancer cluster in marine mammals was reported in adult California sea lions stranded along the California coast. From 1979 to 1994, 66 of 370 (18%) sea lions examined were found to be affected with a poorly differentiated carcinoma of urogenital origin.[54] Further investigation revealed that the lesions arose from genital epithelium in males and females and that the lesions contained deoxyribonucleic acid (DNA) sequences of a novel gammaherpesvirus.[55] The concentrations of PCBs and DDT compounds were higher than those found in seals that died of other causes.[56] This unique sentinel neoplasm appears to arise from an interaction between a herpesvirus and environmental contaminants that may act as immu-nosuppressive agents or from direct DNA damage or tumor promotion.[55,56]

Emerging contaminants

Recently, attention has shifted to a group of persistent organic chemicals termed "emerging contaminants," which include hydroxylated PCBs, polybrominated diphenyl ethers, and perfluorinated compounds. Evidence of toxicity in laboratory animals and widespread exposure among humans has raised concern regarding potential health effects of these and other contaminants found in the marine environment. Recent evidence of high exposure levels in marine mammal populations has also appeared. Studies of bottlenose dolphin populations along the eastern coast of the U.S. show that this sentinel species is highly exposed to brominated flame retardants and their hydroxlyated analogs.[57] Similarly, perfluorinated compounds including perfluorooctane sulfonate were found ubiquitously in dolphins from the Gulf of Mexico and the western Atlantic Ocean.[58] The sampling sites included estuarine environments in the Indian River Lagoon, Florida, and in the vicinity of Charleston, South Carolina. Extensive health assessments have been conducted on these coastal dolphin populations since 2003, under the Bottlenose Dolphin Health and Risk Assessment Project, aimed at investigating associations between dolphin health and environmental contaminants.[42,59] To provide comparative data on health effects and inform human risk assessment, studies of biochemical and endocrine markers, immune function, target organ dysfunction, and health status are in progress.

CONCLUSION

Exciting opportunities exist for exploring marine mammal sentinels of ocean and human health through interdisciplinary research utilizing modern approaches to characterize their effects. The findings from investigations on the effects of environmental pollution on sentinel animals are relevant to human health and may lead to public health interventions and policy initiatives.

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