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Review

Animals as Sentinels of Human Environmental Health Hazards: An Evidence-Based Analysis

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Abstract: Despite recognition that animals could be serving as "sentinels" for environmental risks to human health, there are no evidence-based guidelines for the use of animal sentinel data in human health decision making. We performed a systematic review of the animal sentinel literature to assess the evidence linking such events to human health. A search of MEDLINE identified peer-reviewed original studies of animals as sentinels for either chemical or biological environmental hazards. A limited search of the CAB and AGRICOLA databases was also performed. We classified a random sample of 100 studies from the MEDLINE search according to species, hazard, and health outcome examined; study methods; and linkages to human health. Animal sentinel studies were difficult to locate in MEDLINE because of a lack of adequate key words for this concept. We found significant limitations in the study methods used to investigate animal sentinel events. Clear linkages to human health were frequently absent. Studies of sentinel events in animal populations hold potential for the recognition and control of human environmental health hazards, yet a number of barriers exist to using such data for evidence-based human health decisions. There is a need for greater data sharing and cooperative research between human and animal health professionals regarding environmental hazards and health outcomes in animal and human populations.

Key words: animal sentinel, sentinel surveillance, environmental health, zoonoses, environmental pollution, evidence-based medicine, veterinary medicine, epidemiologic methods

INTRODUCTION

The concept that disease occurrence in nonhuman animal populations (wild and domestic) can serve as a sentinel event warning of an environmental threat to human health has a long and colorful history. Yet 50 years after "dancing cat disease" in Minamata, Japan, presaged the outbreak of methylmercury poisoning in humans and despite the recent linkage between dead crows and West Nile infection in humans, there remain no clear guidelines for the use of animal sentinel data in human health decision making.

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Disease events in animals may also have relevance for ecosystem health in general, but their use specifically for human health (O'Brien et al., 1993) has been advocated in recent years by several national panels (National Research Council, 1991; National Academy of Sciences, 1999; van der Schalie et al., 1999). Requirements for the utility of animal sentinels include predictive reliability and the ability to warn communities of impending danger in time for them to take preventive steps (Stephen and Ribble, 2001). Interest in the potential for animal populations to provide early warning of an attack with biological or chemical warfare agents (Logan-Henfrey, 2000) has led to recommendations for enhanced animal health surveillance (Wilson et al., 2001).

Currently, however, physicians assessing environmental health risks to patients do not routinely include animal sentinel data in their clinical assessments. Public health practitioners are unlikely to respond to mortality events in animals that are not clearly due to West Nile or other known zoonoses such as rabies. Reasons for the underuse of animal sentinel data by human health professionals may include limited understanding of the relationships among animal, human, and ecosystem health; insufficient knowledge of veterinary medicine; and few institutional protocols to incorporate animal data into public health surveillance (Stephen and Ribble, 2001).

Another barrier to such integration may be the relative obscurity of the scientific literature about animal sentinels. Many studies appear in journals not routinely read by medical practitioners or researchers. A previous analysis of the wildlife sentinel literature in MEDLINE encountered difficulties in efficiently locating more than a limited number of sentinel studies by using systematic search techniques (Rabinowitz et al., 1999). Other databases, such as AGRICOLA (the bibliographic database of the agricultural literature created by the National Agricultural Library) and CAB Abstracts may be better geared to animal health events, but they are less frequently used by human health professionals.

For human health professionals to pay more attention to disease events in animal populations, there needs to be a sound scientific argument for a link between such occurrences and human health risks. We therefore set out to examine the evidence in the biomedical literature regarding animals as sentinels for human environmental health hazards.

METHODS

Identifying Animal Sentinel Studies: MEDLINE Search, Inclusion Criteria, and Random Sample Selection

The identification of animal sentinel studies for this research involved four phases. First, to locate animal sentinel studies relevant to human health, we searched the National Library of Medicine's MEDLINE database for the period 1966 to 2002 by using the Ovid search platform. Because there is currently no Medical Subject Heading term in MEDLINE for the animal sentinel concept, we conducted searches separately for two types of sentinel studies: those focused on chemical and physical hazards in the environment and those addressing infectious diseases. Table 1 shows the search terms used in the final Ovid search and the number of MEDLINE citations obtained from each step. For the chemical hazard search, it was necessary to exclude studies of humans to reduce the number of irrelevant citations.

The second phase involved working closely with an interdisciplinary Advisory Board to define inclusion and exclusion criteria for selecting studies as animal sentinel studies from the 5107 citations produced from the MEDLINE search. These criteria were 1) a study of one or more species that were wildlife, companion animals (i.e., pets), or livestock (including poultry); 2) assessment of both environmental exposure and a significant health effect; and 3) relevance of both the exposure and effect to human health (Fig. 1).

The board advised including invertebrates because disease in invertebrates could be relevant to humans, as when clams provide a model for human leukemia (Kelley et al., 2001). For infectious diseases, we focused on examples of animals serving as sentinels by being an alternate host for a disease that affects humans with a common exposure to an infectious agent (e.g., pet dogs in Lyme disease).

The third phase involved two of the authors (P.M.R. and R.H.) independently reviewing the MEDLINE citations for inclusion on the basis of the Advisory Board's criteria. These judgments were compared to arrive at a final sample of 1236 studies (338 of chemical hazards and 898 of infectious disease agents). The final phase involved randomly selecting from these 1236 studies 50 studies of chemical hazards and 50 studies of infectious agents stud-

Table 1. Ovid Search of MEDLINE for Animal Sentinel Studies[a]

Search term	No. of studies
Studies of chemical and physical hazards	
1) exp ANIMALS	583,909
2) Animal	3,428,453
3) Mice	646,432
4) Rats	1,016,720
5) (1 or 2) not (3 or 4)	1,854,665
6) exp Environmental Pollution	156,648
7) (ae or ci or po).fs.[b]	937,305
8) 5 and 6 and 7	7220
9) Human	8,011,318
10) 8 not 9[c]	2912
11) limit 10 to English language	2503
Studies of infectious diseases	
1) Animal	3,428,453
2) Human	8,011,318
3) exp Disease Reservoirs	8149
4) exp ZOONOSES	5369
5) exp ANIMALS	583,909
6) (tm or ep).fs.[d]	570,734
7) (1 or 5) and 6	69,132
8) (3 or 4) and 7	6995
9) 2 and 8	5323
10) limit 9 to English	3384
11) limit 10 to review articles	780
12) 10 not 11	2604

[a]A total of 5107 studies met search criteria.
[b]The search was limited to studies of health effects of these pollutants by using the floating subheadings (fs) "adverse effects" (ae), "chemically induced" (ci), and "poisoning" (po).
[c]Not excluding "human" produced a high rate of irrelevant citations.
[d]Floating subheadings "transmission" (tm) and "epidemiology" (ep) were used.

ies. This produced a random sample of 100 animal sentinel studies for further analysis.

Classification of Animal Sentinel Studies

Each of the 100 studies in the random sample was independently reviewed and classified by a veterinarian (Z.G.) and a physician (P.M.R.). This process involved determining the species, hazard, and health effects studied, as well as the study method and possible links to human health.

A published classification for human epidemiology studies (Kramer and Boivin, 1987) was adapted to create an algorithm for determining the type of method of each study. Figure 1 illustrates the steps in this algorithm. Studies were classified as experimental if the investigator(s) had manipulated exposures. Nonexperimental (i.e., observational) studies were labeled as descriptive case reports if there were no comparisons between groups. Among studies that assessed exposures on an individual level, cohort studies had forward directionality in time, case-control studies had backward directionality, and cross-sectional studies were simultaneous. We determined whether cross-sectional studies analyzed data on the basis of exposure or outcome and whether cohort, case-control, and cross-sectional studies selected controls from the same population as cases or instead from a separate reference population.

For each animal study, the veterinarian and physician determined whether evidence for a link to human health was presented, as shown in Figure 2. Our three inclusion criteria (described previously) had already selected studies of exposures and outcomes relevant to human health. We determined whether such studies documented a shared exposure pathway for both animals and humans (i.e., whether there were data suggesting that humans were also exposed), assessed interspecies differences in susceptibility that would help extrapolate risk from animals to humans, and included any data on human health outcomes related to the hazard in question.

Data Analysis

We looked for time trends in the 1236 studies culled from the initial MEDLINE search by comparing their frequency by year with the total number of citations for that year in MEDLINE. For the 100 studies in the random sample, we entered classification data into a relational database developed for this purpose (a version of this database can be viewed at http://canarydb.med.yale.edu/). Database tables were exported into Microsoft Excel (Microsoft Corp., Redmond, WA), and frequency counts were performed for categorical variables. SAS release 8.02 (SAS Institute, Cary, NC) was used for univariate analyses of continuous variables.

Because we were aware that some animal studies may not appear in MEDLINE, we performed a limited search of the AGRICOLA and CAB Abstracts databases. This search used the Ovid SilverPlatter search platform and the descriptor "sentinel animals," which appears in these databases (but not in MEDLINE). We compared the results of this search with our MEDLINE

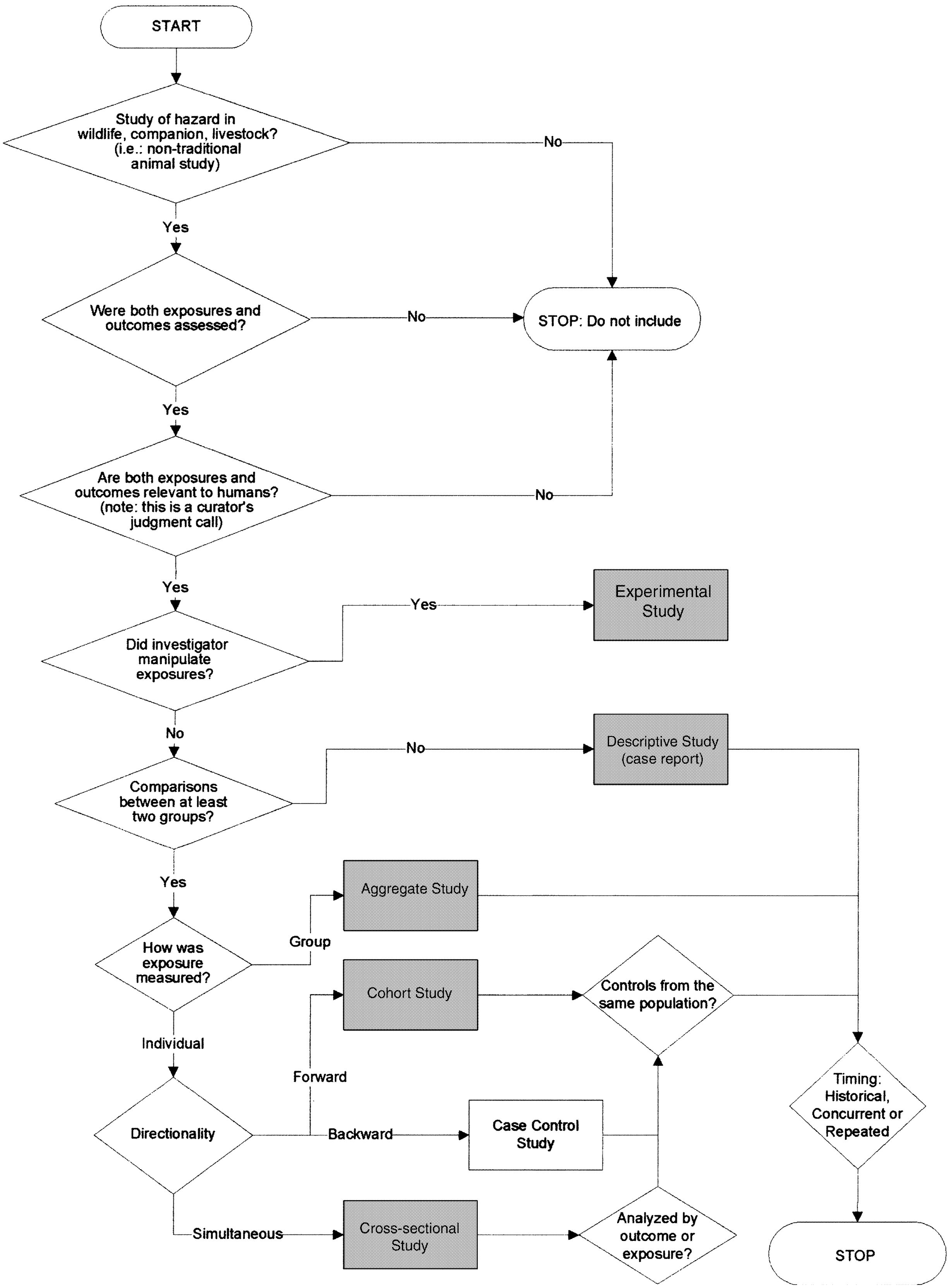

Figure 1. Inclusion criteria and method classification for animal sentinel studies (adapted from Kramer and Boivin, 1987).

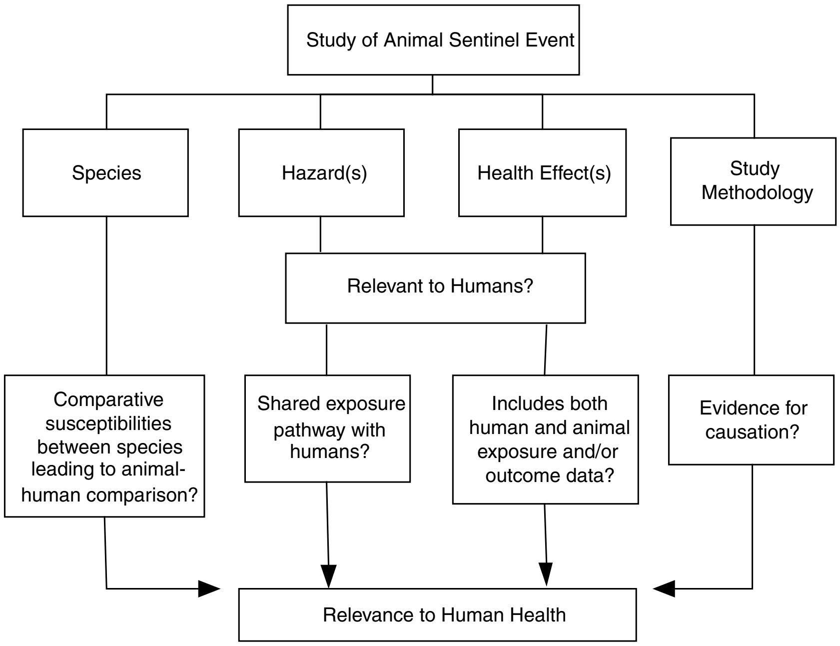

Figure 2. Links between animal sentinel studies and human health.

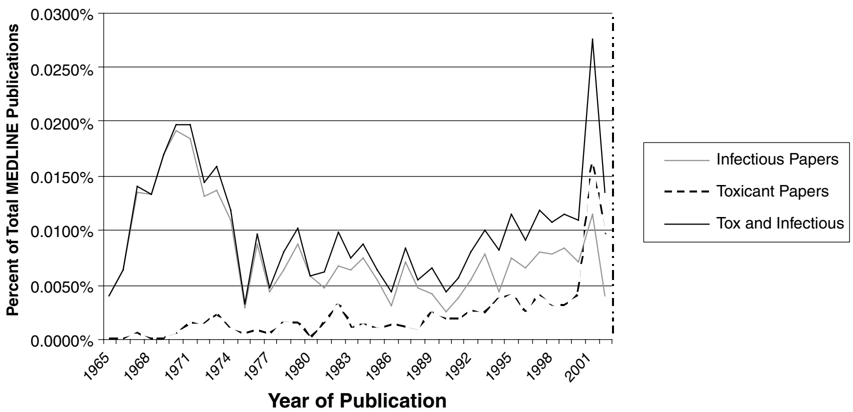

Figure 3. Frequency of animal sentinel studies by year (n = 1236) as a percentage of all MEDLINE citations.

search results to assess the completeness of our approach.

Results

The 1236 animal sentinel studies (culled from the initial MEDLINE search by applying the inclusion criteria defined by the Advisory Board) span a time period from 1966 to 2002. Figure 3 shows the pattern of frequency over time for these studies compared with the total number of citations appearing each year in MEDLINE.

There appeared to have been some periods of increased attention to the distribution of infectious diseases in animal populations during the late 1960s and 1990s. Sentinel animal studies of chemical and physical hazards also apparently increased relative to all MEDLINE citations during the past decade.

Characteristics of Studies Included in the Random Sample

Categories of Sentinel Animals

Table 2 shows the relative frequency of wildlife, companion, and livestock sentinel animals identified in the random sample of 100 studies. Wildlife species appeared most often (94% of chemical/physical hazard studies and 70% of infectious disease studies). Infectious disease studies were more likely than chemical hazard studies to include significant numbers of livestock (34%) and companion animals (24%).

Table 2. Number of Studies by Animal Category, Taxon, and Type of Hazard

Variable	Chemical/physical hazard studies	Infectious disease studies	Total
Animal category			
Companion (pets)	0 (0%)	12 (24%)	12
Livestock	3 (6%)	17 (34%)	20
Wildlife	47 (94%)	35 (70%)	82
Total	50 (100%)	64[a] (128%)	114
Taxon			
Vertebrates			
Birds	13	8	21
Fish	14	1	15
Mammals	11	98	109
Reptiles/amphibians	7	1	8
Invertebrates	11	0	11
Total[b]	56	108	164

[a]Some infectious disease studies included more than one category of animal.
[b]Both chemical/physical hazard studies and infectious disease studies included several species.

Table 2 details the different classes of animals represented. Mammals accounted for 98 (91%) of 108 species in the infectious disease studies. Fish, invertebrates, reptiles, and amphibians were common in the chemical/physical hazard studies but appeared only rarely or not at all in studies of infectious agents.

Hazards and Effects Studied

Table 3 shows the types of hazards studied in the chemical/physical hazard studies. There was a focus on metals and pesticides (35.4 and 17.7%, respectively). Several studies investigated mixed exposures to water pollution due either to sewage effluent or industrial sources.

Table 4 describes the frequency of types of health effects in the chemical/physical hazard studies. There was a wide range of outcomes: reproductive (19.1%), growth and development (12.4%), and immunologic (11.2%) effects occurred most often.

As Table 5 indicates, vectorborne diseases were the most common type of infectious disease in the sample (52.8%), followed by foodborne and waterborne infections (28.1%). The most common infectious pathogens studied were those causing plague and leishmaniasis (six studies of each), followed by the agents for leptospirosis and Hantavirus infection (four studies each; data not shown).

Table 3. Chemical/Physical Hazards Studied (n = 50 Studies)

Hazard	No. of studies	%
Air pollutants—nonspecified	5	5.2
Hydrocarbons/petrochemicals	5	5.2
Metals and related compounds	34	35.4
Organic solvents	1	1.0
Other chlorinated compounds	9	9.4
Pesticides and related compounds	17	17.7
Radiation	1	1.0
Sewage	9	9.4
Water pollutants—nonspecified	15	15.6
Total[a]	96	100

[a]Some studies included more than one hazard.

Table 4. Health Effects in Studies of Chemical and Physical Hazards, by Organ System

Health effects	No. of studies	%
Cancer/genotoxicity	4	4.5
Dental	4	4.5
Dermatologic	2	2.2
Endocrine	2	2.2
Gastrointestinal	5	5.6
Genotoxicity	3	3.4
Growth and development	11	12.4
Hematologic	7	7.9
Immunologic	10	11.2
Mortality	7	7.9
Neurobehavioral	8	9.0
Other/systemic toxicity	2	2.2
Renal	3	3.4
Reproductive	17	19.1
Respiratory	5	5.6
Sensory	2	2.2
Total[a]	89	100

[a]Some studies included more than one health effect.

Methods of Animal Sentinel Studies

Table 6 shows the distribution of study method types for the 100 animal sentinel studies in the random sample. A total of 38% of chemical/physical hazard studies used an experimental design, whereas aggregate (25%) and cross-sectional (23%) designs also appeared frequently. Infectious disease studies were most often cross-sectional (67%), although case reports were also common (14%). No chemical

Table 5. Types of Infectious Disease Studied, by Route of Transmission

Type of infectious disease by transmission	No. of studies	% Total infectious disease studies
Cutaneous	5	5.6
Foodborne/waterborne illness	25	28.1
Multiple route	2	2.2
Respiratory	10	11.2
Vectorborne	47	52.8
Total[a]	89	100

[a]Some studies included more than one agent.

Table 6. Study Methods Used in Sentinel Studies (n = 100)

Study method	Chemical/physical hazard studies	Infectious disease studies
Experimental	20 (38%)	3 (4%)
Observational		
Descriptive case report	6 (12%)	9 (14%)
Analytic studies		
Aggregate (ecologic) studies	13 (25%)	6 (10%)
Cross-sectional studies	12 (23%)	42 (67%)
Analyzed by outcome	1	2
Analyzed by exposure	11	34
Both	0	3
N/A	0	3
Case-control studies	0 (0%)	1 (2%)
Cohort studies	1 (2%)	2 (3%)
Total[a]	52 (100%)	63 (100%)

N/A, not applicable.
[a]Some studies used more than one method.

hazard studies and only one infectious disease study used a case-control approach, and cohort designs were rare in both categories (2 and 3%, respectively).

Experimental Studies

Chemical hazard studies were often experimental and brought animals in to the laboratory, as when researchers tested the effect of lead exposure on the behavior of hatchling slider turtles (*Trachemys scripta*) from a wild population (Burger et al., 1998). One team maintained fish in a tank supplied continuously with water from a polluted river compared with controls raised in tap water (Schmidt-Posthaus et al., 2001). This blurred the distinction between the tightly controlled exposures of a laboratory experiment and the uncontrolled exposures of an observational study.

Infectious disease studies less frequently used experimental designs, often in conjunction with another method. An example was a study of hepatitis E prevalence in swine that performed a survey of infection prevalence and also experimentally infected several pigs (Arankalle et al., 2002).

Descriptive Case Reports

Descriptive case reports were commonly encountered (15% overall). For example, a report on the effect of crude oil on seabirds described several cases without examining any controls (Khan and Ryan, 1991). Similarly, a study of leishmaniasis described cutaneous lesions in two individual rodents only (Morsy et al., 1987).

Aggregate (Ecologic) Studies

Aggregate study methods were often used to study chemical hazards (25%). An example was a study of the effect of sewage on sex hormone levels in fish. The researchers found depressed testosterone in male fish living below a sewage outflow compared with fish from another site, and they attributed the effect to chemicals in the sewage (Folmar et al., 2001). Some aggregate studies were repeated over time, as when pooled samples of organochlorines in eggs and assays of immune function were compared each year for groups of terns (*Sterna caspia*) in a polluted and unpolluted site (Grasman and Fox, 2001).

Cross-sectional Studies

Cross-sectional studies assessed both outcomes and exposures on the individual level at a single moment in time. Many of the infectious disease studies (67%) used a serologic or other diagnostic test to document infection and were therefore classified as cross-sectional because the test determined both the exposure to an infectious agent and the infection. Twenty-three percent of chemical hazard studies also used a cross-sectional design, as when both levels of contaminants and measures of immune function were assessed in an oyster (*Crassostrea virginica*) population (Fisher et al., 2000).

The analysis of data in the cross-sectional studies was almost always performed on the basis of exposure rather than outcome. For chemical hazard studies, this usually involved comparing individuals in a polluted area with individuals in a reference (less polluted) area and measuring differences in outcomes between groups. For example, Yauk et al. (2001) compared lung histopathology in ring-billed gulls (*Larus delawarensis*) living near a point source of air pollution with that of gulls living in a geographically removed area with better air quality. Similarly, infectious disease studies often compared rates of infection in animals living in one geographic area with those in another area to determine risk factors for infection, as when leptospirosis seroprevalence in raccoons (*Procyon lotor*) was compared for populations living in two distinct areas (Mitchell et al., 1999).

Case-control Studies

Case-control studies compared individuals with a certain condition (cases) with individuals without the condition (controls) for evidence of previous exposures (backward directionality). None of the sampled chemical hazard studies used this approach, but a single infectious disease study did. In that study, investigators compared leptospirosis antibodies in a group of clinically ill dogs and controls that appeared well. They found greater evidence of previous leptospirosis infection in the sick dogs (Weekes et al., 1997).

Cohort Studies

Cohort studies compared exposed and unexposed individuals over time (forward directionality) for the occurrence of an outcome. The animal sentinel studies in our sample rarely used this approach. In one example, researchers used enclosure cages to segregate and monitor several populations of fish living different distances downstream from a sewage treatment plant (Mitz and Giesy, 1985). In a study of Japanese encephalitis virus, a sentinel herd of originally seronegative pigs was followed up with serial blood tests to detect new cases of viral infection (Detels et al., 1976).

Sample Size of Animal Sentinel Studies

Table 7 shows the total study sample size for studies in both categories in which sample size was mentioned. Forty-three chemical hazard studies and 47 infectious disease studies included these data. The median sample size was more than twice as great for infectious disease studies (284) as for chemical hazard studies (107), and one infectious disease survey sampled more than 71,000 animals.

Table 7. Study Sample Size[a]

Sample size	Chemical/physical hazard studies	Infectious disease studies	Total
No. of studies[a]	43	47	90
Total no. of animals studied	13,261	124,954	138,215
Median	107	284	139.50
Minimum	1	4	1
Maximum	2000	71,332	71,332
SD	471.96	10,710	7797

[a]For 90 studies reporting a sample size.

Links to Human Health

As Table 8 demonstrates, infectious disease studies often mentioned concurrent exposure of human populations (68%), whereas studies of chemical hazards rarely documented such shared exposures. No chemical hazard studies in the sample presented data on health outcomes in humans, whereas this was common in infectious disease studies (62%). This discrepancy could be due in part to the decision to exclude human studies in the chemical hazard search (Table 1). Infectious disease studies were more likely to include evidence about interspecies differences in susceptibility (18 vs. 4%).

Preliminary Search of CAB and AGRICOLA

The limited search of CAB and AGRICOLA by using the “animal sentinel” key word produced 371 AGRICOLA citations and 213 CAB citations. After application of the same inclusion criteria used for the MEDLINE search, 93 AGRICOLA studies (72 infectious disease and 21 chemical hazard studies) and 91 CAB studies (73 infectious disease and 18 chemical hazard studies) were identified. Reasons for exclusion included a focus on animal sentinels for veterinary rather than human health. Only two of these studies had appeared in the original MEDLINE search, but most journals in which the CAB and AGRICOLA animal sentinel studies appeared are also found in the MEDLINE database.

Table 8. Evidence for Links between Animal Sentinel Events and Human Health Risk

Study type	Animal-human health exposure[a] (%)	Interspecies differences[b] (%)	Inclusion of human outcomes[c] (%)
Chemical hazard	8	4	0
Infectious disease	68	18	62
Total	38	11	31

[a]Includes data on shared exposures in animals and humans.
[b]Includes data on differing susceptibilities between species.
[c]Includes data on links between animal exposure/effects and human health outcomes.

Discussion

This review represents a unique collaboration of animal health and human health professionals to systematically assess the scientific evidence regarding whether animals can serve as useful sentinels of environmental threats to human health. We found that although studies of sentinel events in animals may be appearing in the medical literature with increasing frequency, such studies use a restricted range of study methods (compared with the human epidemiologic literature) that could limit their validity. We also found many differences between animal studies of infectious agents and those examining chemical and physical hazards. The two categories of animal sentinel studies differed in study methods, species, sample size, and the inclusion of evidence linking an animal sentinel event to human health.

It was beyond the scope of this review to confirm or explain our finding that animal sentinel studies may be increasing in frequency in the MEDLINE database. Possibilities include recent concerns regarding emerging infectious diseases, biological and chemical terrorism threats, and abnormal developmental events in animal populations. Despite this apparent increase in scientific activity, the multiple and time-consuming steps that were necessary to locate animal sentinel studies in MEDLINE illustrate a barrier to the sharing of scientific information between animal health and human health researchers. One simple solution could involve introducing a "sentinel animal" Medical Subject Heading term into MEDLINE, as CAB Abstracts and AGRICOLA have done, thereby allowing this literature to gain greater coherence and recognition.

Criteria for successfully proving that an environmental hazard causes an adverse health outcome apply to animal and human studies (Fox, 1991). These criteria include biological plausibility, time order, strength of association, specificity, consistency on replication, predictive performance, and coherence. The study method that investigators choose to investigate an animal sentinel event can have a major effect on the study's ability to provide such evidence, yet our sample found limitations in the range of methods used.

Experimental designs, often used in our sample for studies of chemical hazards, have the advantage of controlling for all variables except the exposure of interest, thus reducing the risk of bias and confounding. Yet in the study of animal sentinel events, limitations of an experimental design include the expense of creating a controlled laboratory environment and maintaining a laboratory population of animals, as well as the difficulty of replicating environmental exposures. Additionally, and most importantly, an experimental trial can test only a very limited set of hypotheses. Therefore, experiments would seem most appropriate only after several observational studies have generated hypotheses worthy of further confirmation.

Case reports, a common method in our analysis, can be useful for generating hypotheses. They are therefore often the first type of study to undertake in the investigation of a suspected hazard or outbreak of disease in an animal population, where it is necessary to describe the outbreak in terms of time, place, and animals affected. Such descriptive reports, however, because of the lack of group comparisons, cannot analyze or measure the strength of the association between an exposure and an outcome.

Aggregate (also referred to as *ecologic*) studies, in which rates of disease are compared between groups from different geographic areas (where exposures are assumed to differ), are also fairly simple to perform and were another common method in our sample. Aggregate studies, unlike case reports, can test hypotheses, yet they are unable to prove causation. Because exposures are not assessed on the level of the individual, there is no way of knowing whether the diseased individuals were actually exposed. Such studies therefore run the risk of the *ecologic fallacy* of asserting, for example, that higher rates of a developmental deformity in animals from one area compared with a population from another site are due to differences in pesticide use, when in fact another contaminant (or other factor) is the culprit (Jekel et al., 2001).

Cross-sectional studies, in which both outcomes and exposures are assessed on an individual level, allow investigators to avoid the ecologic fallacy and, like aggregate studies, may be simple and quick to perform. Our review found that animal studies of infectious disease often used cross-sectional designs, testing individual animals for antibodies or other evidence of infection. Because information on both exposure and effect is available for each subject, cross-sectional data can be analyzed in terms of either outcome or exposure. Analyzing on the basis of outcome involves taking a group of individuals affected by a particular health outcome and comparing them with a group that does not have the outcome. Levels of contaminants or evidence of infectious exposure can then be compared between groups. Conversely, analyzing a cross-sectional study in terms of exposure involves comparing the degree of health effects in a group that has a certain exposure, such as a certain increased level of contaminants, with another group that has a lower level of contaminants. We found that most animal studies of chemical and physical hazards analyzed cross-sectional data on the basis of exposure.

In human epidemiology, the decision of whether to sample and analyze on the basis of outcome or exposure is generally made on the basis of whichever is rarer (Kramer and Boivin, 1987). If an outcome is fairly rare, such as a limb deformity in a frog population, it makes sense to attempt to sample on the basis of outcome, because a sufficient number of cases could be assembled more economically and compared more easily with a number of unaffected individuals than if an entire population had to be sampled. An advantage of this approach is that for a particular outcome, a number of different etiologies can be explored. If, conversely, an exposure is fairly rare, it makes sense to concentrate on the subjects that have that exposure (as in a cohort design) and to look for health effects in this population compared with an equivalent number of individuals who do not have the exposure.

By analyzing cross-sectional data almost exclusively on the basis of exposure (not outcome), the sentinel studies assumed that there was little diversity of exposure among individuals in the "polluted" or "exposed" area and that it was necessary to choose "unexposed" individuals from a separate population to provide unexposed individuals. This separate reference population was often quite geographically removed from the polluted or exposed site. In studies of animal populations, in which natural selection may be exerting different pressures even in populations separated by small distances, there is great potential for selection bias. Furthermore, animals living in a polluted area may have adapted genetically to their environment and may be different from a population living in pristine area (Dickerson et al., 1994; Karels et al., 2001). This selection effect could mean that differences found between populations could have less to do with a particular exposure and more with genetics.

A key weakness of both aggregate and cross-sectional studies of environmental hazards is that they cannot determine facts about the order of exposure and outcome—in other words, whether one preceded the other. Systematic bias can also cloud the results of a cross-sectional study. If a chemical or infection has a fatal effect on animals, a cross-sectional survey will underrepresent the true number of individuals that have been exposed (*late-look bias*). Alternatively, it may overrepresent exposures that have sublethal effects; this is known as *Neyman bias* (Jekel et al., 2001).

Case-control studies, in which exposures are retrospectively assessed in cases and control subjects, are one way to address time order. They are also a good way to study rare diseases that could be missed in a cross-sectional survey. Unlike experimental studies, case-control studies can examine several different risk factors at once. This allows the investigator to adjust for possible confounding variables and efficiently test several hypotheses simultaneously that can later be confirmed in an experimental setting. However, in our sample of sentinel studies, the case-control method appeared only once. Although there are obvious difficulties in reconstructing historical exposures for an animal that cannot fill out a questionnaire, this obstacle is not insurmountable. Tissue samples, otolith analysis, exposure records for an area, and so on can help to create an exposure history. For example, Hayes et al. (1991) used questionnaires of dog owners to calculate historical chemical exposures for dogs that had been diagnosed with lymphoma vs. cancer-free control dogs.

The cohort design, considered the strongest observational design in human epidemiology, was also rare in our sample. The forward directional approach of a cohort study approximates an experimental design. There are challenges to tracking individuals in animal populations over time; yet, again, this should not preclude the use of the cohort method in animal sentinel studies. We found examples of successful use of enclosures or sentinel herds or flocks that allowed investigators to monitor the incidence of disease occurrence in an animal population over a defined study period. Other technologies, such as capture–recapture techniques and electronic tracking devices, could also be used to observe an animal cohort.

Our review could not assess the reasons why animal sentinel studies failed to make greater use of case-control and cohort methods. In humans, these approaches can be more costly and time-consuming than other methods such as the cross-sectional and aggregate study designs. It is therefore possible that funding limitations may have affected choices of animal sentinel study methods.

Another aspect of a study that affects its ability to provide valid evidence is the sample size. We found that studies of infectious agents had larger study samples than studies of chemical and physical hazards. This may reflect the lower cost of testing for an infectious agent compared with performing expensive toxicologic analyses. The development of more economical biomarkers of chemical exposure may make larger studies of toxic exposures more viable. Tests that are less invasive will help to avoid animal welfare concerns. Although it would seem logical to combine the efforts of the infectious disease and toxicology community to ensure that any sampling of an animal population can provide the greatest scientific benefit, the results of our review suggest an apparent division between the infectious disease and toxicology research communities. Most infectious disease studies in our sample did not assess chemical exposures, and vice versa. Animal populations, in fact, may experience exposure to both infections and chemicals, and certain toxins may affect susceptibility to infection (Aguirre and Lutz, 2004). When an animal sentinel event is investigated, it seems worthwhile to collaboratively examine both infectious and noninfectious etiologies.

A key issue with studies of animal sentinels is their actual relevance to human health. We propose a set of linkage points that can be useful in assessing the evidence bridging an animal sentinel event to human health. This process identified gaps in the current knowledge base, such as inadequate documentation of shared exposures, shared outcomes, and relative susceptibility between animals and humans for environmental hazards. These knowledge gaps limit the current usefulness of many animal sentinel studies to human health professionals. It is possible that as the field of comparative genomics expands, there will be more opportunities for such linkages on a molecular level by looking at DNA sequence homology between sentinel species and humans for target genes of interest.

Conclusions

In recent years, there has been a move toward an evidence-based medicine approach in human medicine and veterinary practice (Rosenthal, 2004). The potential for animal data to provide important evidence regarding environmental risks to human health seems obvious. However, the results of this systematic examination of the animal sentinel literature reveal limitations in study methods and a frequent absence of clear links to human health. Solutions include efforts to make the animal sentinel literature more accessible to human health professionals. Another step would be for human health and animal health professionals to collaborate in studies that gather data on environmental exposures and adverse health outcomes in both animal and human populations concurrently. This could lead to a more rapid development of effective study methods for investigating animal sentinel events and could strengthen the evidence base for the use of animal sentinel data in human clinical and public health decision making.

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