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Dallas Virchow
University of Nebraska Panhandle Research and Extension Center

Wayne L. Kramer
Nebraska State Health Department

Charles S. Brown
U.S. Department of Agriculture

Scott E. Hygnstrom
University of Nebraska-Lincoln, shygnstrom1@unl.edu

Alan M. Barnes
U.S. Public Health Service- Centers for Disease Control

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FIRST EVIDENCE OF PLAGUE (*YERSINIA PESTIS*) IN NEBRASKA IS FOUND IN PANHANDLE PREDATORS

Dallas Virchow

Department of Forestry, Fisheries, and Wildlife
University of Nebraska Panhandle Research and Extension Center
Scottsbluff, Nebraska 69361

Wayne L. Kramer

Nebraska State Health Department
P. O. Box 95007
Lincoln, Nebraska 68509

Charles S. Brown

United States Department of Agriculture
Animal and Plant Health Inspection Service
Animal Damage Control
5940 South 58th St.
Lincoln, Nebraska 68501

Scott E. Hygnstrom

Department of Forestry, Fisheries, and Wildlife
University of Nebraska–Lincoln
Lincoln, Nebraska 68583

Alan M. Barnes

Centers for Disease Control
U.S. Public Health Service
Division of Vector-borne Infectious Disease
Fort Collins, Colorado 80522

ABSTRACT

In September, 1992, plague (*Yersinia pestis*) antibody was found in the blood of a coyote (*Canis latrans*) (1:128 titer) and a badger (*Taxidea taxus*) (1:2048 titer) taken near a suspect black-tailed prairie dog (*Cynomys ludovicianus*) town in Sioux County. However, a 1989–1991 survey of 96 coyotes taken from nine Nebraska Panhandle counties demonstrated no plague antibodies. Passive hemagglutination tests of Nobuto blood-sampling paper proved useful to quickly survey a predator population that is distributed across a broad geographic area.

† † †

Plague, caused by the bacterium *Yersinia pestis* (formerly *Pasteurella pestis*) is a flea-transmitted disease of sylvatic rodents that can infect humans. The primary concern to human health involves the transmission of plague-causing bacteria to urban or rural commensal rat (*Rattus* spp.) populations, which increases the potential for human infections (Hudson and Quan, 1975). Another important concern is secondary pneumonial infection that can occur among humans and the subsequent pneumonic transmission to other humans. Cases of human plague and secondary plague pneumonia have gradually increased in recent years in the United States (Barnes, 1990; Smith et al., 1984).

Plague-infected humans living in tropical climates exhibit a form of the disease characterized by enlargement of lymph nodes that appear as "buboes" on the skin, giving it the name "bubonic" plague. Severe hemolysis, during bubonic infections, can cause a darkening of the skin that evidently inspired the name "black death" during the severe plague outbreak that occurred in 16th-Century Europe.

Nelson has characterized the major routes of sylvatic plague transmission as vector, oral, and pneumonic (Nelson, 1989). The latter route is probably the most common mode between conspecifics. Cannibalism among rodents can transmit plague via ingestion (Barnes, 1982).

Native wild rodents and their fleas are the primary source of human infection, although other wild and domestic animals can play intermediary roles. Predators can become exposed to plague infection by consuming infected rodents or by being bitten by infected fleas (Barnes, 1982). There is some risk to humans when they handle or skin predator carcasses (Von Reyn et al., 1976).

Outbreaks of plague among wild rodents have been

located in foci across the western United States (Fig. 1). Numbers of plague-positive individuals within such foci may periodically increase dramatically and the infection may expand to nearby geographic areas and to other species, including humans. Primary plague foci in the United States include the Rocky Mountain and High Plains regions of Colorado and Wyoming, the Colorado plateau area of Colorado, New Mexico, and Arizona, and the Pacific coastal area from California into southern Oregon (Burnett, 1984).

From 1974 to 1976, a regional epizootic occurred in the Rocky Mountains and High Plains of Colorado and Wyoming. Populations of the Richardson ground squirrel (*Spermophilus richardsoni*) and the golden-mantled squirrel (*Spermophilus lateralis*) were affected. The outbreak also affected other mammals including several chipmunk (*Eutamias*) species, tree squirrels (*Sciurus aberti* and *Tamiasciurus hudsonicus*), deer mice (*Peromyscus maniculatus*), and certain predators such as marten (*Martes americana*), long-tail weasel (*Mustela frenata*), and coyote (*Canis latrans*).

The present study was conducted to determine the presence of plague and plague antibodies in the Nebraska Panhandle. Although plague has been identified in all Wyoming and Colorado counties that border the Nebraska Panhandle, it was yet to be observed in Nebraska at the time of our study (Fig. 2). The study also sought to provide supportive information for human health and safety and for the potential reintroduction of the black-footed ferret in Nebraska. Allan M. Barnes, in an unpublished report, *A review of plague (*Yersinia pestis*) infections and its relevance to prairie dog populations and the black-footed ferret*, established parameters for the selection of reintroduction sites for black-footed ferrets (*Mustela nigripes*). Such sites include large, plague-free prairie dog complexes.

METHODS

Nobuto's blood-sampling papers (Toyo Roshi Kaisha, Ltd., No. 7 3-Chome, Hon-Cho, Hihonbashi, Chuo-ku, Tokyo, Japan) were used to collect samples from 40, 44, and 12 coyotes in the Nebraska Panhandle in 1989, 1990, and 1991, respectively. During September, 1992, blood-sampling papers were again used to sample one coyote and one badger taken within 500 meters of a black-tailed prairie dog town in southern Sioux County. In this town, plague activity was strongly suggested because prairie dog numbers had declined to zero during the preceding months of July and August. Six thirteen-lined ground squirrels (*Spermophilus tridecimlineatus*) and one cottontail rabbit (*Sylvilagus auduboni*) were also taken from the prairie dog town and sampled by this technique.

Because of the occurrence of ground squirrels and rabbits, flea vectors of the plague bacterium were thought to remain in the prairie dog burrows. Fourteen fleas were taken from ten burrows near ground squirrel or rabbit observations. The samples consisted of 13 *Oropsylla hirsuta* and one *Thrassis fotus*, both common flea species of prairie dogs. Flannel cloth was used to swab prairie dog burrows for fleas, as described by Barnes et al. (1972). Fleas were placed in glass vials of 2% NaCl and .001% Teen 80 and shipped to the Centers of Disease Control (CDC) Laboratory in Fort Collins, Colorado, for processing.

Both sexes of coyote were well-represented within our collections. Twenty-three males and 17 females comprised the 1989 collection. Eighteen males and 23 females made up the 1990 collection, with six unidentified as to sex. Four males, five females, and three unidentified coyotes comprised the 1991 collection. Juveniles represented 4, 36, and 0 percent of the samples in 1989, 1990, and 1991 respectively. Animal Damage Control specialists of the Animal Damage Control Division, United States Department of Agriculture–Animal and Plant Health Inspection Service, captured the coyotes using traps, snares, and sodium-cyanide devices in response to landowner requests for assistance to control livestock depredation.

We used Nobuto's blood-sampling papers, following the manufacturer's recommendations and as used and described by Wolff and Hudson (1974). The sampling papers consist of a 5 × 30 mm section for adsorption of 0.1 ml of whole blood or serum and a 10 × 18 mm section for support during collection and drying. Animal Damage Control Specialists dipped the 5 × 30 mm section into coyote blood, shook it gently to remove excess fluid, and air-dried the paper before placing it in an envelope.

Sex, age, general health or appearance of the animal, and date and location of capture were recorded on the envelopes. Age was determined by incisor wear and body size. Envelopes were kept out of direct sunlight and periodically delivered to the University of Nebraska Panhandle Research and Extension Center, Scottsbluff, then mailed to the Nebraska State Department of Health, Lincoln. There, field data were transcribed and samples were forwarded to the Centers for Disease Control (CDC) Laboratory in Fort Collins, Colorado, for processing. Samples taken from the prairie dog town in Sioux County were collected during a seven-day period in September 1992 by University of Nebraska and Animal Damage Control-Nebraska personnel and immediately sent to the CDC.

Samples were eluted at the CDC laboratory by cutting the 5 × 30 mm section in half and extracting overnight at 4°C. Blood samples were immersed in 0.4

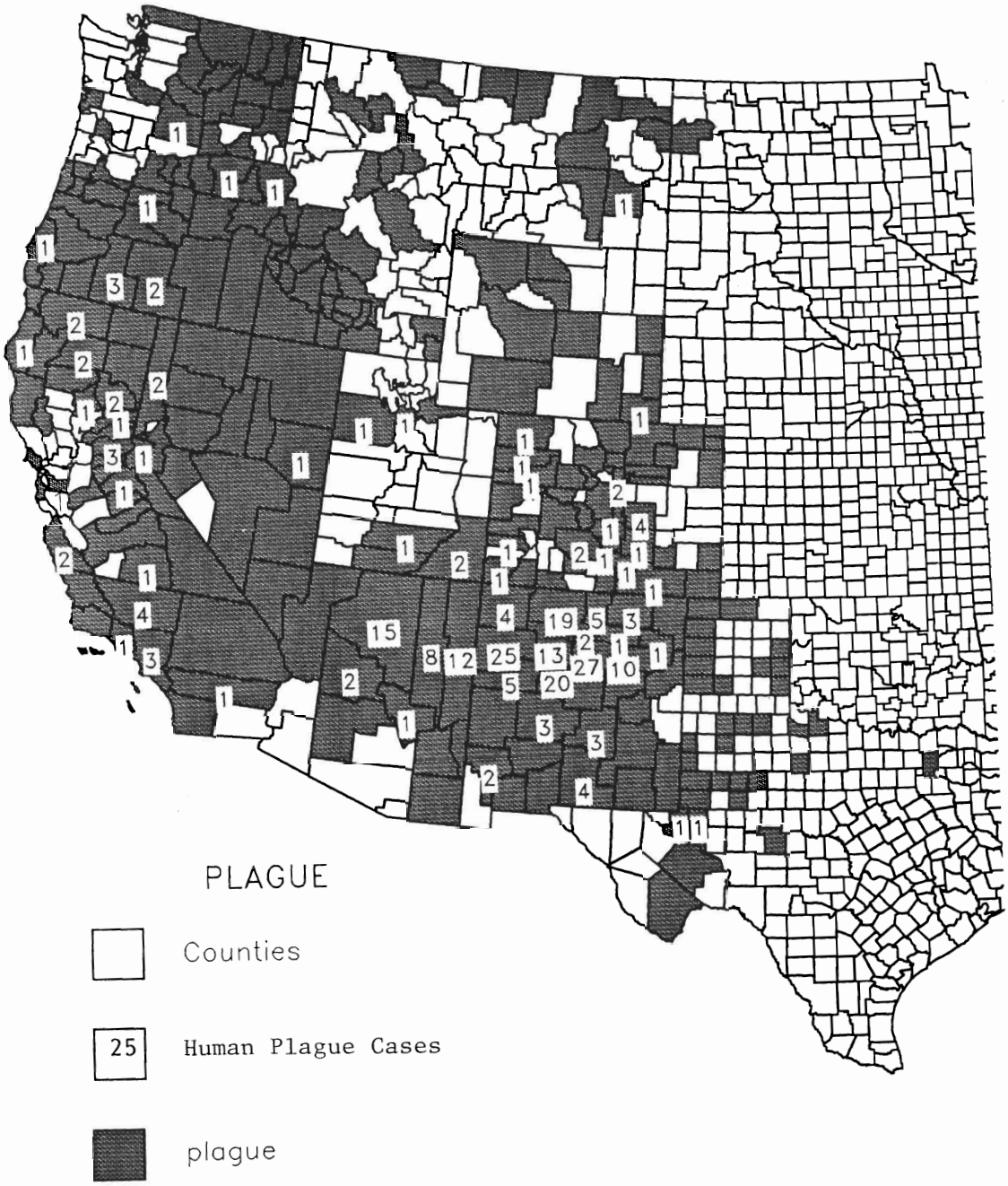

Figure 1. Geographic distribution of human and animal plague in the United States by county of origin 1970–1986 (taken from Barnes, 1990).

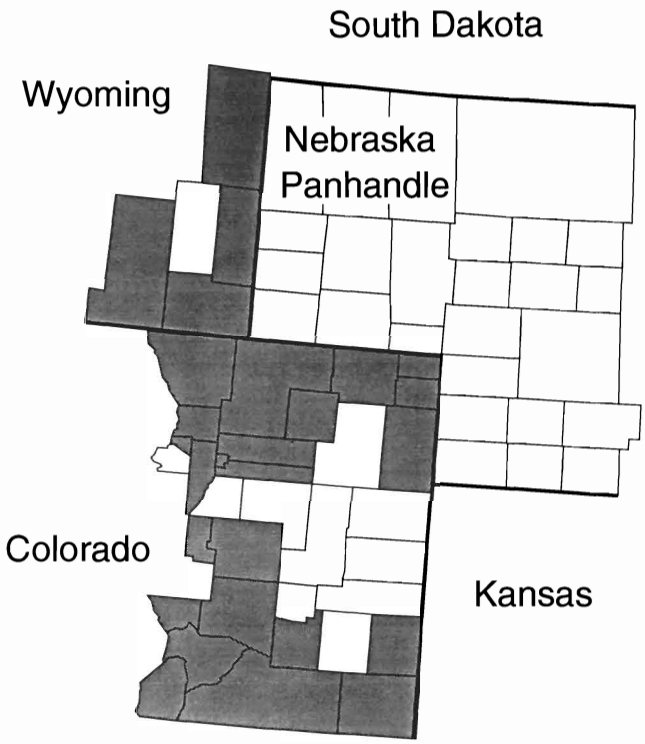

Figure 2. Geographic distribution of human and animal plague adjacent to Nebraska by county of origin (adapted from Barnes, 1990).

ml of borate buffer at pH 8.0. Vials containing the diluent and paper strip were inactivated at 56°C for 30 minutes. The paper strip was pressed to the bottom of the vial with a glass rod and the extract was adsorbed with washed sheep erythrocytes (1:10, vol/vol) for 20 minutes at room temperature. After centrifugation, the supernatant fluids were tested for the presence of antibody to the water-soluble fraction 1A envelope protein of *Yersinia pestis*. Wolff and Hudson (1974) used this method and noted that a comparison of titers for whole blood strips yielded consistent results within the limits expected for the passive hemagglutination method.

RESULTS AND DISCUSSION

One coyote and one badger taken near a suspect prairie dog town in southern Sioux County had titers of 1:128 and 1:2048 for the plague antibody. These titers represent low and very high levels, respectively. Both titer levels provided strong evidence for recent plague activity, probably within months prior to sampling (T. Quan, CDC, pers. comm.).

All thirteen-lined ground squirrels, the rabbit, and the fleas sampled at this site were seronegative. Also, none of the 96 coyote-blood samples taken from nine counties of the Nebraska Panhandle from 1989 to 1991 tested positive for the plague antibody. These sample locations were concentrated in the northern Panhandle counties of Sioux, Dawes, and Sheridan, but all Panhandle counties, excluding Garden and Deuel, were represented (Fig. 3).

The Sioux County prairie dog town where seropositive samples were found is a remote location. In July 1989, USDA APHIS Animal Damage Control personnel were requested by the landowner to control the prairie dog population. USDA APHIS Animal Damage Control records indicate that the prairie dog town was about 90 hectares and averaged 57 burrows per acre. Zinc phosphide-treated oats were used after a prebait application. A subsequent census indicated greater than 90 percent reduction in prairie dog numbers (Don Fryda, USDA APHIS Animal Damage Control, pers. comm.).

In July 1992, again at the request of the landowner, USDA APHIS Animal Damage Control personnel visited the site to determine need for management of the prairie dog population. The population had grown to 62 hectares and averaged 24 burrows per acre. July observations indicated normal numbers of prairie dogs, but no prairie dogs could be found in a subsequent survey of the area in mid-August. Their disappearance prompted an intense sampling effort in early September 1992. During this effort, no prairie dogs were seen, but thirteen-lined ground squirrels and cottontails appeared in normal numbers.

Small mammals, other than prairie dogs, are often unaffected by plague outbreaks in prairie dog colonies (Clark, 1977; Lechleitner et al., 1968; Ubico et al., 1988). Fleas also are commonly seronegative for plague, despite their presence in prairie dog burrows on infected colonies. Ubico et al. (1988) sampled 165 burrows in an infected white-tailed prairie dog town in northwestern Wyoming and found 52% of burrows with fleas but only 15% of burrows with plague-positive fleas.

Not enough is presently known about flea ecology in prairie dog towns to adequately predict plague behavior in prairie dogs and their predators. Numbers and proportions of flea species can vary from colony to colony and from season to season.

In contrast with our seropositive findings in the Sioux County prairie dog town, a pair of suspect prairie

dog towns in Box Butte County provided no evidence for plague. In January 1992, our survey collected twelve coyotes within 8 km of two suspect prairie dog towns. The prairie dog towns were about 12 and 20 hectares in area. Normal populations of prairie dogs had been seen on each site in spring, 1991, and clipped vegetation had little regrowth when the areas were revisited in December, indicating prairie dogs were present throughout most of the growing season (J. Wall, USDA APHIS Animal Damage Control, pers. comm.). Blood samples collected from coyotes during January 1992 showed no evidence of plague antibody.

Plague may not have been in the Box Butte County area but a delay in sampling could have affected results. Plague can quickly eliminate prairie dog colonies, sometimes within months (Barnes, 1982; Cully, 1987; Raynor, 1985). Because blood titers may persist for only a few months, predator blood should be taken from individuals near suspect prairie dog colonies as soon as possible after a colony die-off. Also, predators less resistant than coyotes may succumb to the disease without transmission to conspecifics.

Serological studies have found significant numbers of animals in wild carnivore populations to carry the antibody. A survey of wild carnivores, domestic dogs, and cats was made from 1976 to 1980 in 15 western states. It found 13.6% of 10,296 coyote samples to be seropositive for plague antibody (Barnes, 1982). The

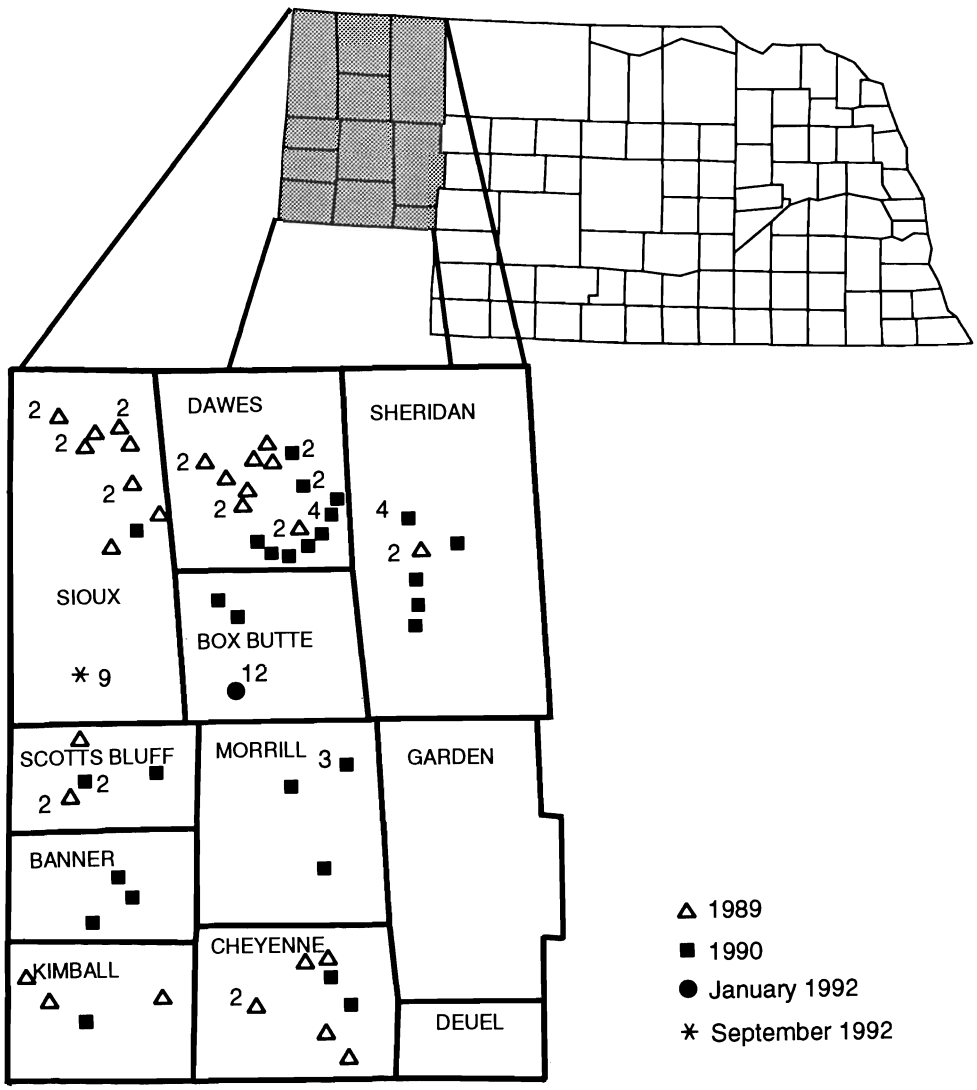

Figure 3. Distribution of coyote blood samples collected in the Nebraska Panhandle during the summers of 1989, 1990, January, 1991, and September, 1992. Numerals indicate number of samples at that location.

broad geographic area sampled included both states with periodic epizootics and those without such occurrences. Coyotes represented 83% of all wild carnivore samples tested in that survey. The values of wild carnivores that tested seropositive ranged from 2.3% in Washington to 27.9% in Arizona.

In a less extensive study, a coyote population was sampled in a high sylvatic-plague area of California (Willeberg et al., 1979). Twenty-one percent of the 143 coyotes sampled tested seropositive for the plague antibody . Despite the positive serologies of the coyote and the badger at the Sioux County site, we are relatively confident that a high sylvatic plague area does not currently exist in Nebraska's Panhandle, given the much higher incidence of plague among predators found in these other studies.

Carnivore-serum methodology has been used with success in other studies (Archibald and Kunitz, 1971; Taylor et al., 1981). Cruickshank et al., (1976) used carnivore-serological studies to delineate spatial and temporal plague distribution in Africa. In Idaho, badgers were used to monitor plague in the Townsend ground squirrel (*Spermophilus townsendi*) (Messick et al., 1983).

Canids have proven to be especially useful monitors of plague because they produce a quick response to infection and show antibodies to *Y. pestis* for several months (Rust et al., 1971). When using the passive hemagglutination test, onset of detectable coyote titers occurs at 8 to 14 days, peaks at 20 to 30 days and persists for 6 to 8 months (T. Quan, CDC, pers. comm.). Other predators, such as bobcats, show relatively high incidence of the plague antibody but also succumb more easily to the disease than coyotes. This decreases their usefulness as a monitor species. Canids, however, often show little clinical sign of the disease, which makes them good blood test subjects. However, this trait also makes field observation alone an insufficient tool to monitor plague.

Studies have shown that serological tests for carnivores provide a simple way to detect persistence of plague both within foci and over broad geographical areas and to delineate plague epizootics (Smith et al., 1984). We believe that monitoring predator blood for plague has advantages over monitoring rodent blood. Fewer predators than rodents are required because predators can consume many infected and noninfected rodents and other animals. In addition, we believe that the mobility of the coyote distinguishes it as the best predator to use in studies that encompass broad geographic areas like the Nebraska Panhandle. Barnes and others proposed that coyotes provide indirect evidence for plague in rodent populations, even during inter-epizootic periods, given their response to the disease (Barnes, 1982).

Wolff and Hudson (1974) noted that the paper-strip technique for collection fails to detect animals with low titers. However, they noted several advantages that counterbalance such failings. For widespread serological surveys, methods of collection and handling are simple and little personnel training is required. Amounts of blood required are small (0.1 ml). Road-killed or poisoned animals or animals killed in deadfall traps can be used. Access to laboratory facilities and refrigeration is not required, so dried specimens can be mailed in envelopes via normal postal delivery.

The contrasting results between the Box Butte and Sioux county sites illustrates the need to quickly survey potential plague activity areas. We believe Nobuto's blood-sampling paper works well under these restraints.

MANAGEMENT IMPLICATIONS

Now that plague has been identified in Nebraska, a public education program must be developed to increase awareness of the potential public health problem. The Nebraska State Health Department and the University of Nebraska Cooperative Extension have begun to directly contact physicians, informing them of the incidence and symptoms of plague. A media program is currently being developed to alert high-risk individuals and to inform the general public via tempered news releases.

The Nebraska Game and Parks Commission plans to survey prairie dog colonies in 1992 in the Nebraska Panhandle to determine suitability of areas for reintroduction of black-footed ferrets (*Mustela nigripes*). The presence of plague would significantly hinder reintroduction efforts. We believe the Nobuto's blood-sampling-paper technique would fit well into a responsive program to monitor plague.

The Nobuto's blood-sampling paper technique proved useful to quickly sample coyote blood collected from across the Nebraska Panhandle and to spot-check suspect plague activity sites. We believe that any comprehensive program to monitor plague should include blood samples taken from carnivores across a broad geographic area. We recommend that coyote blood be used to sample from such areas and that both rodent and predator blood be used in local suspect areas, such as at dying or recently abandoned prairie dog towns.

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