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First Detection of *Bacillus anthracis* in Feces of Free-ranging Raptors from Central Argentina

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ABSTRACT: Prevalence of anthrax spores in feces of raptors was determined from samples collected in November–December 2000 and April–May 2001 in an agricultural region of Santa Fé province, Argentina. Feces were tested from 48 birds of six raptor species. One of 14 chimango caracaras (*Milvago chimango*) and one of eight road-side hawks (*Buteo magnirostris*) tested positive. The prevalence of *Bacillus anthracis* spores in feces for the six species was 4% ($n=48$). The prevalence was 7% ($n=14$) for chimango caracaras, 13% for road-side hawks ($n=8$), and 0% for the remaining species (Burrowing owl [*Speotyto cunicularia*] [$n=17$], Swainson's hawk [*Buteo swainsoni*] [$n=3$], Aplomado falcon [*Falco femoralis*] [$n=2$], and American kestrel [*Falco sparverius*] [$n=4$]). Grouped by their feeding habits, prevalence for scavenger species was not significantly different than for predators (7% vs. 3%, $P>0.999$). This study provides evidence that in central Argentina scavenger and non-scavenger raptors may have a role in the epidemiology of anthrax. Long-term studies to determine the extent of this potential involvement in the epidemiology of anthrax in central Argentina are required.

Key words: Anthrax, Argentina, *Bacillus anthracis*, raptors.

Anthrax, caused by *Bacillus anthracis*, is a per-acute to acute infectious disease that mainly affects domestic and wild herbivores; it has a high mortality rate (Berry, 1993). Captive and free-ranging carnivorous mammals and birds also can be affected (Hugh-Jones and de Vos, 2002). The epidemiology of anthrax is highly complex, involving interactions between wild herbivores, predators, scavengers, insects, domestic livestock, strain characteristics, and environmental factors (de Vos, 2003).

The role of wildlife in the epidemiology of anthrax has been mostly studied in North America (Dragon et al., 2001) and Africa (de Vos, 1990; Lindeque and Turnbull, 1994). Anthrax is also known to have a role in the ecology of a number of free-ranging wildlife species in many other parts of the world (Hugh-Jones and de Vos, 2002). Among raptors, scavenger species, such as New World (Cathartiformes) and Old World vultures (Accipitriformes), have been considered as disseminators of anthrax spores, which they can carry on their beaks, body, feathers, and feet (Bullock, 1956; Hugh-Jones and de Vos, 2002). Feces are thought to be the most important mode of transmission (de Vos, 1990), and species that travel long distances may disseminate spores to likely areas where transmission can occur such as watering holes (Hugh-Jones and de Vos, 2002). However, there are few studies on the prevalence of anthrax spores in feces of wild raptors. Lindeque and Turnbull (1994) found anthrax spores in 50% of the feces of three species of vultures (*Gyps africanus*, *Torgos tracheliotus*, *Trigonoceps occipitalis*) at Etosha National Park, Namibia; these samples were collected near confirmed anthrax carcasses. In

Chile, anthrax spores have been recovered from the feces of black vultures (*Coragyps atratus*) and turkey vultures (*Cathartes aura*), but prevalence was not provided (Bullock, 1956).

In Argentina, anthrax affects livestock and humans, was reported as early as 1609 (Noseda et al., 2002), and is known to affect the economy of the region severely (Noseda et al., 1995). One hundred and forty-four livestock outbreaks were reported between 1990 and 2000, with most outbreaks involving cattle but rarely pigs or horses (Noseda et al., 2001). Eighty-three cases of human anthrax were reported between 1977 and 2000, with two fatalities (Noseda et al., 2001, 2002). Although anthrax commonly occurs in livestock in central Argentina, no information has been published on the prevalence of anthrax in wildlife in this area. Several species of diurnal raptors are found in the pampas of central Argentina, and many of these species feed on carrion (del Hoyo et al., 1994), including animals that have died from anthrax (Noseda et al., 2001). We hypothesized that raptors may play a role in the epidemiology of this disease in the pampas region of central Argentina. The aim of this study was to determine the prevalence of *B. anthracis* in the feces of raptors (scavenger and predator birds of prey), and to evaluate their possible role as carriers and disseminators of this infectious agent in Argentina.

Raptors were sampled in November–December 2000 and April–May 2001. The area selected for this study represented a typical agro-ecosystem of central Argentina, modified landscapes that have replaced the region's native grasslands. One study area was located in the Melincué lagoon basin (33°42′S, 61°32′W) in southern Santa Fé province. The region is >70% agricultural. Cattle ranching predominates in locations close to the lagoon and related wetlands, while in the higher areas intensive agriculture predominates. The second study site, Las Petacas (31°45′S, 62°11′W) in central-west Santa Fé province, is mostly utilized for agriculture. Soy, corn, sunflower, and alfalfa are the main crops in this region, and cattle ranching is also important. During the period of study, anthrax was not reported by local farmers or local veterinarians.

Raptors were trapped using Bal-Chatris traps (Bloom, 1987) with live mice, chicks, and finches as lures. For scavengers, noose carpets placed on dead animals were also used. Raptors species trapped during this study were classified as predators (birds that mainly hunt live prey) and scavengers (birds that are predominantly carrion eaters), based on published feeding habits (del Hoyo et al., 1994) and the authors' knowledge.

Fecal samples were obtained by gentle swabbing of the bird's cloaca using a sterile, saline-impregnated, cotton swab. Swabs were immediately embedded in Stuart Transport Medium (Laboratorio Britania, Buenos Aires, Argentina) avoiding contamination and refrigerated at 4 C during transport to the laboratory. Samples were then incubated for 30 min at 65 C in their own transport medium to inactivate Gram-negative bacteria and to select anthrax spores. Heat-treated and untreated samples were cultured in 5% ovine blood agar (Laboratorio Azul, Azul, Buenos Aires, Argentina) and polymyxin-lysozyme-EDTA-thallous acetate agar (PLET, Laboratorio Azul), a selective medium for the isolation and identification of *B. anthracis* (Knisely, 1966). Compatible colonies were tested for motility, catalase reaction, gelatin liquefaction, and penicillin sensibility for preliminary identification. All samples considered positive were confirmed by API 50 CHB (Bio Merieux SA, Marcy L'etoile, France) and identification assisted by APILAB PLUS (Bio Merieux SA). The isolates compatible with *B. anthracis* were inoculated in albino mice (Charles River CF1) with 0.3 cc of inoculum, subcutaneously, with a density of approximated 3×10^5 bacteria/ml. One mouse was not inoculated with spores to serve as a control. Mice were

TABLE 1. Species, number, and percentage of individuals trapped and found positive or negative for *Bacillus anthracis* spores in Santa Fé, Argentina.

Species	FH[a]	*n*	Positive/Negative	Prevalence
American kestrel (*F. sparverius*)	P	4	0/4	0
Aplomado falcon (*F. femoralis*)	P	2	0/2	0
Burrowing owl (*S. cunicularia*)	P	17	0/17	0
Chimango caracara (*M. chimango*)	S	14	1/14	7%
Road-side hawk (*B. magnirostris*)	P	8	1/8	13%
Swainson's hawk (*B. swainsoni*)	P	3	0/3	0
Totals		48	2/48	4%
Scavengers		14	1/14	7%
Predators		34	1/34	3%

[a] FH = Feeding habits: P = predators, S = scavengers.

observed every 6 hr for clinical signs until death. At necropsy, the inoculation sites and the spleens were observed, and *B. anthracis* recovered for isolation and identification. Strain determination was achieved following the technique described by Keim et al. (2000).

Forty-eight individuals of six species were sampled and tested for anthrax spores (Table 1). Of the 14 chimango caracaras (*Milvago chimango*) tested, one was positive. This was an adult of undetermined sex and a body weight of 260 g, trapped in the Melincué area along with six other raptors during a 7-hr period (28 November 2000) using a dead pig as lure. One of eight road-side hawks (*Buteo magnirostris*) tested was found positive for anthrax spores. This bird was judged to be an adult male based on size and body weight (360 g). Both birds were considered in good health status based on physical examination and body weight.

The total prevalence of anthrax spores for the 48 raptors tested was 4%. The prevalence was 7% for chimango caracaras, 13% for road side hawks, and 0% for the remaining captured raptors (Burrowing owl [*Speotyto cunicularia*], Swainson's hawk [*Buteo swainsoni*], Aplomado falcon [*Falco femoralis*], and American kestrel [*Falco sparverius*]). Regarding their feeding habits, prevalence for chimango caracara (scavenger) and road-side hawk (predator) was not statistically significant (Irwin-Fischer test, -0.05, $P>0.999$).

Both isolates were obtained on PLET selective medium, with morphology and biochemical tests compatible with *B. anthracis*. Identification by API 50 CHB showed 98.8% probability of the isolates for this bacterium. Mice inoculated with the isolates died within 26 and 32 hr. Mice had yellowish and gelatinous edema at the inoculation site. Necropsy showed liver and spleen enlargement, and pure *B. anthracis* were recovered from these organs. Both strains belonged to the A genotype and A3a subtype of *B. anthracis* according to genetic analysis.

To the authors' knowledge this is the first report of *B. anthracis* spores in feces of raptors from Argentina. Both scavenger and nonscavenger species had spores in their feces. Even though the prevalence of infection was low, the presence of spores still raises the possibility that raptors may be disseminators of anthrax in this region.

The road-side hawk is a small hawk that usually hunts small vertebrate prey such as frogs, reptiles, rodents, and invertebrates from a perch (del Hoyo, 1994). This hawk has not been reported as a carrion eater. It is possible that anthrax spores were ingested secondarily via some prey that had ingested or touched contaminated animals, or by contact with other species at water sources. The road-side hawk is a sedentary, usually solitary or paired

species, maintaining its territories year round. No information is known about migratory habits or dispersion patterns. It is probable that the role of this raptor as an anthrax disseminator is low because of its biological habits, but further studies are required.

Chimango caracaras are opportunistic and generalist feeders. Though little is known about their biology, they are very sociable raptors, with colonial nesting and communal feeding habits (Salvador and Fraga, 1986). They are usually seen feeding in groups on road-killed and dead farm animals in central Argentina. Chimango caracaras are one of the most common species of raptors in this region and were commonly observed in our study area feeding on dead animals. Despite recommendations to burn or bury livestock carcasses, most carcasses are left in the fields to be consumed by predators and scavengers like chimango caracaras; this may represent the primary source of anthrax spores for chimango caracaras and other scavengers.

Large numbers of chimango caracaras were seen feeding at the unattended dead pig carcass for several days. It is possible that the positive bird picked up anthrax spores while feeding on this lure. The fact that the other six chimango caracaras trapped at this site were negative would tend to negate this individual carcass as a source of infection. According to the owner, this pig showed progressive weakness, loss of weight, and decreased appetite for several weeks before it died, signs that do not support anthrax as cause of death. When grossly examined the pig did not have lesions compatible with anthrax (hemorrhagic pharyngeal lymphnodes). The cause of death of this pig was not further investigated. This carcass, left unattended in the field, represented a real situation in many central Argentina ranches and provided the opportunity to evaluate natural exposure to a potentially infectious dead domestic animal among wild raptors.

The role that scavenger raptors like chimango caracaras, as carrion eaters, play in the epidemiology of anthrax is unclear. Studies conducted in other scavenger species, *G. africanus*, in Africa show that the ingestion of sporulated bacteria is necessary for dissemination, as the vegetative form does not survive transit through the digestive tract (Houston and Cooper, 1975). Vultures contribute significantly to the disappearance of large mammal carcasses in the wild, by rapidly removing a large percentage of their biomass in a few hours (Houston, 1985). De Vos (1974) and Lindeque and Turnbull (1994) suggest that in some cases vultures can eat dead animals before most of the vegetative form of *B. anthracis* has had time to sporulate and thereby reduce the number of infective spores (resistant form) released into the environment. Thus the effect of these scavengers feeding on animals dead from anthrax may be more beneficial than detrimental.

Chimango caracaras do not appear able to consume large dead mammals as efficiently as Old World vultures. They are small birds, with a small and powerless beak, unable to open the skin of medium-to-large-sized mammals. One possibility is that chimango caracaras are exposed to spores when arriving at carcasses *after* they have been opened by another scavenger. In the absence of other large scavenger species that could remove a large mammal like a cow in less than 24 hr, it is possible that chimango caracaras may ingest sporulated *B. anthracis* and contribute to its dissemination. Chimango caracaras are considered migratory, moving to northern latitudes in the austral winter (Zalles and Bildstein, 2000), which could allow them to disseminate anthrax to other areas.

The most important factor regarding wildlife, livestock, and human exposure to anthrax spores is the disposal of dead animals suspected or confirmed to have died of anthrax. One study in Buenos

Aires province showed that carcass disposal was carried out in only 79% of cases; 22% of carcasses were transported (spreading secretions on the soil), 57% were burned, and 43% were skinned for use of the hides (Noseda et al., 2001). Failure to bury infected carcasses properly is most likely an important means of anthrax transmission and perpetuation in central Argentina farmlands. The use of controlled burial techniques has been proposed to eliminate infected bodies, which decreases exposure of wildlife, like chimango caracaras, as well as livestock and humans (Noseda et al., 2002) to anthrax spores.

This study provides evidence that in central Argentina, scavenger and nonscavenger raptors may disseminate anthrax spores in their feces and play a role in the epidemiology of anthrax. Long-term studies should be conducted to determine the extent of wildlife involvement in the epidemiology of this disease in central Argentina, and based on these results, raptors may be important to include in such studies.

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