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ANESTHESIA AND BLOOD SAMPLING OF WILD BIG BROWN BATS (*EPTESICUS FUSCUS*) WITH AN ASSESSMENT OF IMPACTS ON SURVIVAL

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ABSTRACT: We anesthetized and blood sampled wild big brown bats (*Eptesicus fuscus*) in Fort Collins, Colorado (USA) in 2001 and 2002 and assessed effects on survival. Inhalant anesthesia was delivered into a specially designed restraint and inhalation capsule that minimized handling and bite exposures. Bats were immobilized an average of 9.1±5.1 (SD) min (range 1–71, n=876); blood sample volumes averaged 58±12 μl (range 13–126, n=718). We randomly selected control (subject to multiple procedures before release) and treatment (control procedures plus inhalant anesthesia and 1% of body weight blood sampling) groups in 2002 to assess treatment effects on daily survival over a 14-day period for adult female and volant juvenile bats captured at maternity roosts in buildings. We monitored survival after release using passive integrated transponder tag detection hoops placed at openings to selected roosts. Annual return rates of bats sampled in 2001 were used to assess long-term outcomes. Comparison of 14-day maximum-likelihood daily survival estimates from control (86 adult females, 92 volant juveniles) and treated bats (187 adult females, 87 volant juveniles) indicated no adverse effect from anesthesia and blood sampling (juveniles: χ^2=22.22, df=27, P>0.05; adults: χ^2=9.72, df=18, P>0.05). One-year return rates were similar among adult female controls (81%, n=72, 95% confidence interval [CI]=70–91%), females treated once (82%, n=276, 95% CI=81–84%), and females treated twice (84%, n=50, 95% CI=74–94%). Lack of an effect was also noted in 1-yr return rates of juvenile female controls (55%, n=29, 95% CI=37–73%), juveniles treated once (66%, n=113, 95% CI=58–75%), and juveniles treated twice (71%, n=17, 95% CI=49–92%). These data suggest that anesthesia and blood sampling for health monitoring did not measurably affect survival of adult female and volant juvenile big brown bats.

Key words: Anesthesia, bats, blood, *Eptesicus fuscus*, marking effect, PIT tags, survival.

INTRODUCTION

Wildlife health monitoring practices are crucial for assessing disease risk, to infer and model the efficiency of transmission routes, to characterize the role of disease in population dynamics, and to facilitate disease prevention practices. To make reliable inferences about the dynamics of populations being monitored for health status, it is critical to establish the degree to which capture, handling, and sample collection procedures may affect survival (Wobeser, 1994). In addition, ethical and conservation-motivated concerns have encouraged development of techniques that minimize the impact of intervention while assuring maximum returns from monitoring the health of wild populations (Michener, 1989; Bekoff, 1995). In field studies, adverse outcomes may occur during relatively routine procedures such as capture, restraint, transport, anesthesia, tissue sample collection, marking, and release (Kock et al., 1987; Michener 1989; Bekoff, 1995). Anesthesia and blood sampling procedures are commonly performed on bats in captivity and in the field (Gustafson and Damassa, 1985; Wilson, 1988). However, the impacts of these and other disease investigation procedures on subsequent survival of wild bats have not been examined. In the present study, we developed protocols for anesthesia and blood sampling of big brown bats (*Eptesicus fuscus*) that also un-

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derwent other procedures during health monitoring studies. We then determined the impact of these techniques on short-term survival and 1-yr return rates of the bats.

MATERIALS AND METHODS

Collection sites and dates of study

All procedures were approved by the Institutional Animal Care and Use Committee at Colorado State University, Fort Collins, Colorado (USA). Big brown bats were captured as part of an ongoing investigation of rabies ecology in this species that began during summer 2001 and continued during summer 2002. Bats were captured at roosts used by maternity colonies. Roosts were discovered by radio tracking bats initially caught while foraging. All roosts were in buildings in or near Fort Collins, Colorado (UTM coordinates 0492798E 4493203N; 40°35′N, 105°5′W), including churches, barns, commercial buildings, and private homes. Maternity colony sites selected for sampling were based on potential for bat capture and monitoring as well as owner cooperation. Bats sampled at these sites consisted of adult females and volant male and female juveniles (most adult males do not frequent maternity roosts). Roosts where bats were observed for the 14-day short-term survival study were monitored for single 2-wk intervals with collection dates between 10 June and 30 July 2002 (collection dates varied by roost). Bats used to determine yearly return rates were sampled and marked in summer 2001 and detected alive by passive hoop readers during summer 2002.

Bat capture and release

Standard techniques for capturing bats were used (Kunz et al., 1996) and included mist nets, hand-held nets, harp traps, and nylon funnel traps set at evening emergence points at maternity roosts. Capture was typically performed during the early evening emergence of bats (i.e., 8:00–10:00 PM) starting at dusk, when ambient temperatures were moderate and adult bats were effectively in a fasted state. Contact among bats was avoided during all handling procedures. Once removed from nets or traps, bats were placed in small, prewashed cloth geological specimen bags (Hutchinson Bag Corporation, Hutchinson, Kansas, USA) closed with a drawstring. Bags containing bats were kept individually separated in 0.5-l disposable paper cups with lids. Each cup containing a bat was then placed in a plastic cup-holding rack, and transported to a central sample processing area at Colorado State University, requiring <30 min of transport time. Racks of bats were placed ~3 cm above standard electric heating pads on the floor (to aid blood sampling via vasodilation, and to prevent induction of torpor). People handling bats wore leather gloves to prevent bites. All personnel handling bats received pre-exposure prophylactic immunizations for rabies or annual tests to determine anti-rabies virus serum antibody titers. Leather gloves were sprayed with 95% ethanol or were covered with new latex gloves before handling each bat. Bats were released simultaneously at the original collection site within 6 hr of capture.

Sample collection

Each bat was removed from its bag and demeanor, body condition, age, sex, and reproductive status were determined. Each bat was closely scored for number and distribution of bite wounds or scars on wings and ears as a measure of recent aggressive encounters (O'Shea, 1980). Forearm length was measured to the nearest 0.1 mm using dial calipers. Body weight was determined in preweighed cloth bags to within 0.1 g (Pesola spring balance AG, Baar, Switzerland). Two sterile cotton swabs (Fisher Scientific, Pittsburgh, Pennsylvania, USA) dipped in virus isolation medium (MEM-10) were inserted into the mouth to collect samples for rabies polymerase chain reaction screening, using a shortened 1 cc tuberculin syringe barrel as a mouth speculum. Using aseptic technique, a full-thickness round 4-mm-diameter punch biopsy was collected from the plagiopatagium (caudomedial wing membrane) for DNA analysis for genetic studies (Worthington Wilmer and Barratt, 1996). Bats were assigned to adult or juvenile age categories on the basis of the degree of fusion of the phalangeal epiphyses (Anthony, 1988). Additional sampling procedures were added in 2002, when bats were also photographed and visually inspected for number and type of ectoparasites. Also in 2002, a subset of bats captured for the first time were marked by application of a small (3–4 mm) circular freeze-brand applied to the prescapular area (Sherwin et al., 2002). Freeze branding produces a small patch of permanently white pelage used to estimate PIT (passive integrated transponder) tag losses.

Passive integrated transponder tagging

Each bat was implanted with a PIT tag (Avid Inc., Norco, California, USA) for individual identification (Barnard, 1989). Before application, the pelage was trimmed at the insertion point and aseptically prepared. Sterile PIT tags

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FIGURE 1. A hoop passive integrated transponder (PIT) reader in position to detect PIT tag-carrying bats. The roost exit point is largely hidden above and behind the hoop. Transiting bats possessing subcutaneous PIT tags prefer to pass through the hoop and are recorded. Hoops like this one were used to derive 14-day apparent survival estimates and 1-yr return rates.

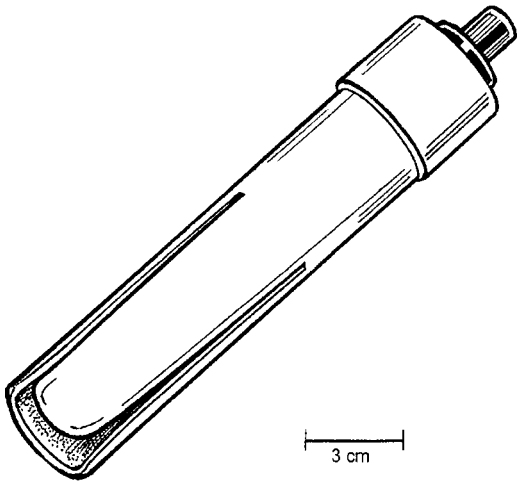


FIGURE 2. The drawing shows a capsule used to restrain and anesthetize bats and to reduce potential bite exposures during manipulations. Either 2.2-cm- or 2.75-cm-diameter polyvinyl chloride pipe (10 cm long) was used for smaller (e.g., *Myotis* species or young *Eptesicus*) or larger bats (adult *Eptesicus*) respectively. One end of the pipe had a glued-on cap and was drilled and fitted for an anesthetic machine coupling that was fixed in place with epoxy. Parasaggital slots were cut to a depth of approximately two-thirds the length of the long axis. All cut edges were rounded and sanded until smooth.

were inserted subcutaneously over the lower lumbar region using a single-use disposable syringe presterilized by the manufacturer. The insertion point was sealed with Nexaband® tissue glue (Closure Medical Corporation, Raleigh, North Carolina, USA). The 0.06 g, 12 × 2.1 mm PIT tags emit an instantaneous (0.04 sec) 125 kHz signal with a unique nine-digit code when they pass within about 15 cm of an activating reader. The presence of individual PIT-tagged bats was passively recorded as they exited and returned from selected roost entrances through permanently positioned circular hoop activating antennas (Figure 1; NEMA readers, Avid, Inc., Norco, California, USA). A 12-V battery-powered data logger was attached to the antenna as part of the NEMA reader system. The data logger stored the digital bat individual identification code, date, and times of detection. These data were downloaded to laptop computers three times each week, and returned to the laboratory where they were entered into a standard query language database. Bats held in hand were scanned for PIT tags using a hand-held Power Tracker IV reader (Avid).

Anesthesia and blood sampling

We developed procedures for sampling blood from bats under gas anesthesia because preliminary experience suggested that sampling in the absence of anesthesia appeared to delay hemostasis, caused prolongation of restraint, and had the potential for increased exposures of personnel to bites. Anesthesia for blood sampling was accomplished by placing each bat in an elongate capsule constructed from either 2.75 cm or 2.2 cm (smaller bats) inner diameter polyvinyl chloride plumbing pipe 10-cm long with a sealed airtight cap on one end (Fig. 2). Sampling a bat under general anesthesia with appropriate handling precautions is shown in Figure 3. A hole was drilled at the center of the end cap to accommodate an endotracheal tube fitting for attachment to an isoflurane nonrebreathing patient circuit (Mapleson E, MWI Veterinary Supply Co., Denver, Colorado, USA). The tube edges were rounded and smoothed at the open end of the capsule where the bat was inserted. On either side of the capsule, starting from the open end, a parasaggital-oriented slot (4.1 mm wide by 5 cm long) was made to generously accommodate each extended bat wing up to the level of the shoulder when the body of the bat was inserted inside the capsule. Once the wings above the elbows were passed through the slots, each bat could be immobilized inside the capsule by gently holding its wings against the outside of the tube. To induce general anesthesia, isoflurane was delivered from a calibrated precision vaporizer through a six-way balanced splitting

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FIGURE 3. A big brown bat restrained in the anesthetic induction capsule with engorged left interfemoral vein. Blood is collected using a 75-μl heparinized hematocrit tube after lancing the vessel with a 27-gauge needle (inset).

manifold. Thus, six separate patient circuits, each with their own endotracheal fitting and anesthetic induction capsule, allowed anesthetic induction and maintenance of up to six bats simultaneously. Initial induction consisted of 4–5% isoflurane and 1 l/min oxygen delivered per bat. Anesthesia was maintained at 2–3.5% isoflurane, using the same oxygen flow rate. Each capsule was disinfected with alcohol between uses. Collection of blood was made by initially warming the site with a hot water bottle until interfemoral vein engorgement was evident in the tail membrane (Fig. 1). A 27-gauge needle was used to lance the interfemoral vessel, typically from the dorsal aspect. Blood was collected into multiple heparinized hematocrit tubes (75 μl; Fisher Scientific, Pittsburgh, Pennsylvania, USA). The goal was to collect under 1% of body weight in blood volume equivalent from each bat. In some cases, bilateral vessels were accessed to guarantee an adequate sample. Hemostasis was accomplished by applying direct pressure with cold packs, or rarely by application of hemostatic gel (Cauter-Gel, Mensa Products, Fort Lauderdale, Florida, USA). Bats were observed until recovery, then placed in their holding bags and cups after completion of any additional sampling. Bats were then transported to the original capture site for release at the same time. During release, bats were allowed to grasp onto a raised gloved hand until they took flight on their own during release. Sometimes supplemental heat from the heater in the transport vehicle was required to insure adequate warming before release.

Study design and analysis

Bats were captured as they exited maternity roosts after sunset. For the 14-day short-term survival study conducted in summer 2002, a random number table was used to assign bats to two groups during each capture. Controls received all procedures except anesthesia and blood sampling, whereas the treated group was also anesthetized and blood sampled. Daily apparent survival rates were computed over the 2 wk subsequent to treatment on the basis of Cormack–Jolly–Seber (CJS) models using Program MARK (White and Burnham, 1999), a numerical maximum-likelihood program devel-

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oped to estimate parameters from mark-recapture data. Only the first capture and handling event for each individual was used in this analysis. Daily presence or absence ("capture-recapture") records of bats passively recorded by PIT tag readers at roosts over the 14-day period subsequent to the night of sampling were used to compute short-term daily apparent survival and capture probabilities. Short-term daily survival estimates were reported as *apparent* survival rates ($\hat{\phi}$) because recapture procedures could not distinguish bats that actually died from bats that emigrated, used roosts without passing over readers, or lost tags. Fourteen-day records of daily bat presence and absence were combined across six roosts for adult females and across five roosts for juveniles of both sexes. Data history files for the two distinct age groups were analyzed separately. Goodness-of-fit (χ^2) testing using Program RELEASE (Burnham et al., 1987) was used to determine differences in apparent survival between treated bats and controls for each of the two age groups, using an alpha of 0.05.

To investigate the potential impact of one or more anesthetic procedures and blood sample collections on long-term fate of bats, 1-yr return rates to maternity roosts were calculated for adult and juvenile female bats that were first sampled and marked with PIT tags in 2001. Passive hoop reader PIT tag detection records at seven roosts monitored in 2002 were used to determine if these bats had survived and returned to the same roost 1 yr after sampling. Bats sampled at these roosts were categorized as PIT-tagged and otherwise sampled but not anesthetized or bled in 2001, or as anesthetized and bled once in 2001 or twice in 2001. Because this was a retrospective analysis, individuals were assigned to groups fortuitously sampled. Bats sampled for blood a second time in 2001 were sampled when recaptured after a minimum of at least 2 wk had elapsed since the initial administration of anesthesia and blood collection. One-year return rates ($\hat{r}$ = number known alive in 2002/number captured in 2001) were calculated separately for adult and yearling females (yearling or adult males do not return to maternity roosts) within each of these groups, with confidence intervals for $\hat{r}$ calculated on the basis of an estimated binomial variance = $\hat{r}(1-r)/n$ (Williams et al., 2002).

RESULTS

Blood samples were obtained from big brown bats under isoflurane anesthesia on 84 nights in 2001 and 2002. These included samples taken from 827 adult females, 248 juvenile females, and 210 juvenile males. Times recorded for 876 immobilizations under anesthesia averaged 9.1±5.1 min (mean±SD; range 1–71). Collection volumes measured for 718 samplings averaged 58±12 μl of blood (mean±SD; range 13–126), excluding extravasation. No anesthetic difficulties were observed, and no bats died during sampling as a result of these procedures. Use of anesthesia and induction capsules reduced the potential for humans to be bitten, reduced handling stress, facilitated blood sampling, and worked well in all bats regardless of age. We had the subjective impression that bats that were aroused from torpor immediately before induction of anesthesia appeared to be slower to induce and to recover. However, warming and continued stimulation were sufficient for a complete recovery in these individuals.

Short-term daily survival rates did not differ between control and treatment groups (Table 1). Lack of an effect of anesthesia and blood sampling on this measure of short-term survival was evident in both adult females ($\chi^2=9.7$, df=18, $P=0.94$) and juveniles of both sexes ($\chi^2=22.2$, df=27, $P=0.73$). Estimates of capture probabilities were high with low variance using this method (Table 1). One-year return rates of bats bled under anesthesia once or twice in 2001 were comparable to each other and to return rates of bats captured and sampled but not bled under anesthesia; all three groups in both age classes had broadly overlapping confidence intervals (Table 2), suggesting that these procedures also had no measurable effect on long-term outcome.

DISCUSSION

There is increasing interest in developing field approaches for assessing the impact of disease on wild animal populations, particularly in wildlife susceptible to anthropogenic influences (Daszak et al., 2001). Big brown bats typically cohabit buildings with humans (Barbour and Davis 1969; Kunz and Reynolds 2003); thus they

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TABLE 1. Maximum likelihood estimates of daily apparent survival ($\hat{\phi}$) and capture probabilities (p) for adult female and juvenile big brown bats over the 14-day period subsequent to capture and handling categorized as treated (anesthetized and blood sampled) and control (not anesthetized, not blood sampled) groups. Estimates were based on passive detection of the presence or absence of individual bats by PIT tag readers placed over roost entrances during summer 2002.

Age	Treatment (n)	$\hat{\phi}$ (95% CI)	p (95% CI)
Adult	Treated (n=187)	0.984 (0.977–0.989)	0.757 (0.738–0.775)
	Controls (n=86)	0.958 (0.942–0.978)	0.757 (0.725–0.785)
Juveniles	Treated (n=87)[a]	0.968 (0.954–0.978)	0.636 (0.604–0.668)
	Controls (n=92)[b]	0.956 (0.940–0.968)	0.660 (0.626–0.692)

[a] 44 juvenile males, 43 juvenile females.
[b] 47 juvenile males, 45 juvenile females.

may serve as an important indicator species for monitoring transfer of diseases to or from humans, and effect of human influences (such as excluding bats from their roosts) on the likelihood of disease transmission. In field studies, disease-induced mortality may be overestimated when surveillance involves capture and intensive sampling that could have an adverse impact on survival. For example, wing banding, the most common marking system used to identify wild bats during past capture-recapture and census studies, has been shown to cause injury and to affect survival estimates in wild bats, even when performed by experienced personnel (Herreid et al., 1960; Humphrey and Kunz, 1976; Baker et al., 2001). Studies performed with terrestrial snakes suggest that PIT tags in adults and young had no appreciable effect on survival or growth (Keck, 1994; Jemison et al., 1995). Our study involves the first use of PIT tagging for capture-recapture estimation of survival in wild juvenile and adult big brown bats using passive detection methods. Combined with continuous collection of presence-absence information from passive hoop readers, this technology further improves survival estimates by reducing the potential effect of avoidance of standard capture techniques by experienced bats, a key source of bias in estimation of survival that has been documented in some studies of bats (Stevenson and Tuttle, 1981; Tuttle and Stevenson, 1982).

The estimates of short-term daily survival and 1-yr return rates by passive monitoring of PIT-tagged big brown bats clearly indicate that the anesthesia and blood sampling techniques we applied to our study population had no measurable impact on mortality. The 1-yr return rates we observed (66–71% for volant juveniles; 82–84% for adult females) were comparable to or exceeded cruder estimates of annual survival of big brown bats, even though these previous reports did not involve collection of multiple biological samples. Banded big brown bats sampled in summer at two maternity colonies in roosts in buildings in Ohio had return rates of 28% to 71% at one site and 10% to 70% at a second (Mills et al., 1975). Brenner (1968) reported that adult female big brown bats sampled at another roost in Ohio returned at rates of 53% in 1 yr and 24% in another, with juvenile return rates of 17% and 32%; adult females sampled at a colony in Pennsylvania returned at rates of 21% in 1 yr and 10% in another, with juvenile return rates of 12–14%. Ad hoc estimates of annual survival for this species made over a 15-yr period at two summer colonies in Arizona ranged from 71% to 90% per year for banded adult females

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TABLE 2. One-year (2001 to 2002) return rates (S) for female big brown bats handled for most sampling procedures but not anesthetized and blood sampled (controls), or also sampled for blood under anesthesia on one occasion or two occasions in 2001 (treated). One year return rates were calculated based on passive detection of the presence or absence of individual bats by PIT tag readers placed over roost entrances during summer 2002.

Age	Treatment (n)	$S \pm SE$	95% CI
Adults	Controls (n=72)	0.806±0.047	(0.698, 0.881)
	Treated once (n=276)	0.822±0.023	(0.773, 0.863)
	Treated twice (n=50)	0.840±0.052	(0.711, 0.918)
Juveniles	Controls (n=29)	0.552±0.092	(0.372, 0.719)
	Treated once (n=113)	0.664±0.044	(0.572, 0.745)
	Treated twice (n=17)	0.706±0.110	(0.458, 0.872)

and 53% to 64% for juveniles (Sidner, 1997). Other estimates of annual survival of big brown bats have been made on the basis of recaptures at hibernacula, and these also are lower than or comparable to the 1-yr return rates we observed (Goehring, 1972; Hitchcock et al., 1984). However, the lower survival estimates reported may reflect the superiority of PIT tagging and passive detection techniques over past methods of marking and capturing bats. Other factors also differed between our study and past efforts that may also affect these comparisons.

Entwistle et al. (1994) conducted the only other study we are aware of that examined the potential impact of invasive sampling techniques on insectivorous brown long-eared bats (*Plecotus auritus*) in Scotland. Unfortunately, methods in that study were different enough from our study to make meaningful comparisons difficult. Although bats are likely to vary by species in their susceptibility to stress during disease monitoring, their dependence on flight to feed, their support of rapidly developing dependent young, and their likely dehydrated state when leaving maternity roosts in summer might predispose them to diminished survival after multiple procedural interventions. Similarly, blood sampling reduces circulating blood volume, oxygen-carrying capacity of the blood, and causes local damage, possibly significant to a flying species that typically catches its food in its interfemoral membrane. Use of anesthesia during blood sampling may have had the attendant benefit of reducing blood pressure and facilitated hemostasis without the excessive stress of continued restraint, or the application of potentially caustic hemostatic agents. Isoflurane–oxygen anesthesia appeared to minimize residual effects commonly observed with other anesthetics used in the field, leading to successful winged releases, and high survival.

We are uncertain about the extent to which our findings can be extended to other species of bats. There have been no similar studies of other species of bats reported and only a limited number of studies on effects of anesthesia and blood sampling on CJS estimates of survival of terrestrial small mammals. Effects of anesthesia and blood collection on survival of small mammals can vary by species. Swann et al. (1997) determined monthly survival and return rates in a nine-species assemblage of small desert rodents that were sampled for blood under methoxyflurane inhalant anesthesia in southeastern Arizona. No treatment effect was noted except in the two smallest species, pocket mice (*Chaetodipus baileyi* and *C. penicillatus*), which had significantly lower survival after anesthesia and sampling of blood. Similarly, Parmenter et al. (1998) sampled 11 species of small rodents from New Mexico in treatment groups subject to sampling for both blood and saliva under methoxyflurane anesthesia. They reported no effect of anesthesia and sampling on mortality or capture probabilities of most of these small

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mammals, with the notable exception of some heteromyids. Unfortunately, the physiologic or pharmacologic reasons why species vary in response to capture, anesthesia, and sample collection are not well understood. Studies involving other bat species would be desirable to ascertain the possible variation in responses to anesthesia and blood sampling within this large order of animals.

In conclusion, anesthesia and blood sampling methods as we describe can be used without adversely biasing evaluations of disease impacts on either short-term survival or 1-yr return rates in wild adult female and volant juvenile big brown bats. Aside from obvious humane advantages, the techniques used here should help to improve the reliability of disease impact estimates in bat populations of importance to conservationists, wildlife disease experts, and public health officials.

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