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Topical Review

Veterinary Assessment for Free-Ranging Eurasian Black Vulture (*Aegypius monachus*) Chicks in Southeastern Mongolia

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Working as a veterinarian in remote field locations can be physically and intellectually challenging. A collaborative multi-disciplinary approach is often required for successful data collection. Technologies and methodologies frequently need to be modified to work in these harsh field environments. This article will describe a collaboration in southeastern Mongolia collecting blood for sera analytes and physiologic data from Eurasian Black Vulture (*Aegypius monachus*) chicks during a tagging operation.

Veterinarians can provide valuable expertise in collaborative field conservation efforts. Yet, veterinary evaluations conducted in the field present many unique challenges not found in domestic animal or zoo practices. Working in a field "bush" camp is often physically challenging, requiring a high level of physical fitness and an ability to endure harsh environmental conditions. Obtaining permits to collect, export, and analyze biomaterials in foreign laboratories can be daunting and time consuming. An alternative is to set up a laboratory in-country and perform analyses in the field obviating the need for export permits. However, setting up and running a functioning field laboratory also presents a number of challenges. Some methodology typically used in the controlled environment of a hospital laboratory may fail in the field, requiring innovative adjustments. In addition to limited portability for laboratory equipment, some of these sensitive devices may require stringent environmental operating conditions. Similarly, sample preparation may require a controlled environment; i.e., temperature, humidity, etc. Advances in point-of-care technology like the ESA Biosciences LeadCare I blood lead analyzer and the i-STAT portable clinical analyzer have helped make field-based laboratory analyses for free-living species a reality. Access to a power source for operating laboratory equipment may prove perplexing, but advances in solar and wind power technology can provide reliable power in habitats with consistent sunshine or wind. But despite these difficulties, veterinarians and veterinary technicians can become valuable collaborators with biologists and also contribute original medical research.

Since 2002, the Denver Zoological Foundation in collaboration with the Mongolian Academy of Sciences has managed a year-round research camp at the Ikh Nart Nature Reserve (hereafter Ikh Nart [IN]) in southeastern Mongolia. Research in IN takes a total ecosystem holistic approach as opposed to a single-species approach. In addition, research at IN uses a highly collaborative approach integrating several different scientific disciplines. Research topics include botany, wildlife ecology, archeology, social sciences, and veterinary medicine. This complex, collaborative effort has provided research opportunities not only for primary investigators but also for domestic and foreign students. Veterinarians, veterinary technicians, and Mongolian veterinary students have assisted biologists in IN with animal captures and animal care issues and also conducted original research.

The Eurasian black vulture has a Palearctic distribution ranging from the Iberian Peninsula in the west through the Balkans, Turkey, Caucasus, Iran, and Afghanistan to northern China, Central Asia, the Korean Peninsula, and the Russian Far East.[1,2] The age of the first breeding has been reported to be 5-6 years.[3] In Mongolia, adults construct massive stick nests on rocky outcrops and ledges, or occasionally in trees.[4] The females lay a single egg in late February to early March and the parents regurgitate the scavenged meat from wildlife and the more abundant livestock for nestlings. Most chicks in Mongolia remain in the nest until the end of August to early September when fledging occurs. Ikh Nart (IN) appears to provide an important breeding area for this species. Our free-ranging Eurasian black vulture (*Aegypius monachus*) project in southeastern Mongolia is an example of an ecosystem approach and interdisciplinary collaborative project performing basic health assessments and the construction of reference ranges involving students, biologists, veterinarians, and veterinary technicians in the field.

Study Area

IN lies approximately 300 km southeast of the capital city Ulaanbaatar. Established in 1996, IN protects 43,740 ha of open valleys and large mazelike rocky outcroppings in northwestern Dorno-Gobi-Aimag.[5] IN is a high upland habitat (altitude ~1200 m) covered by semiarid steppe vegetation. Permanent cold water springs are available in some of the several shallow, sandy valleys draining the reserve. The climate is strongly continental and arid, characterized by cold winters (low −40°F, −40°C); dry, windy springs; and relatively wet, hot summers (high +109°F, +43°C). Precipitation is low and seasonal, with most occurring in

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the summer. Birdlife International designated IN as an Important Bird Area in 2004 because of the high nesting density for Eurasian black vultures, which are listed in Appendix II of the Convention on the International Trade in Endangered Species.

Our team (Denver Zoological Foundation [DZF] and Mongolian Academy of Sciences) located active nest sites starting in late February and follow these active nests monthly until the chicks fledged. Since 2005, DZF has provided a veterinary presence to assist with chick capture, patagial tagging, and morphometric measurements. As we handle 30-40 chicks annually, we decided that this afforded us an excellent opportunity to conduct comprehensive health assessments and collect biomaterials for analysis in the field to establish benchmark reference ranges for this free-ranging population. Beginning in 2009, we collected blood for serum lead analysis and feces for fecal examinations. In 2010, we expanded our efforts to include complete blood counts, serum chemistries, and venous blood gas measurements. In addition, we monitored the physiological and environmental parameters at capture. We considered all chicks that we included for this project to be healthy.

We captured chicks from 2 sites: IN (permanent research camp N 45.72318, E 108.64502) and Chair Mountain (CM, base camp N 46.2530, E 108.78042). We used Global Positioning System units to locate active nests each spring. Most nests occurred on rocky outcrops or ledges (~60%), but many breeding pairs used Russian elm trees (*Ulmus pumila* [~40%]). We collected blood from vulture chicks in IN from 2009 to 2012 and in CM from 2009 to 2010. We stopped capturing chicks in CM in 2011 because only 1 active nest remained, and we expanded our work into the southern regions of IN, reducing our ability to effectively work 2 sites. We do not know why breeding activity declined.

Sample Collection

Chicks were approximately 3-4 months old when captured. We captured chicks between early morning (~7 AM) and early afternoon (~2 PM) to avoid the hottest parts of the day. We hand-captured, manually restrained, and hooded chicks to minimize stress following capture. We quickly moved the chicks to a shady area or created shade with a vehicle. For venipuncture, we placed chicks in dorsal recumbency and extended a wing to expose the ventral cutaneous ulnar vein at the level of the elbow. We did not hold off the vein (to obtain a free-flowing peripheral venous sample) to avoid interference with artificially created acid metabolites, which could negatively affect blood gas results and minimize damage to cells, for hematological analysis.[6,7] It helps for visualization of the vein to pluck some of the feathers and then moisten the area. At our permanent research camp in IN, we established 1 ger (Mongolian round tent) as a dedicated office or laboratory with solar- and wind-generated power. At CM and the extreme southern part of IN, we used a large tent as a portable field laboratory.

In 2009, we obtained approximately 0.7-mL blood sample using a syringe and 25 gauge needle for a purple-top tube with ethylenediaminetetraacetic acid (EDTA) (250-500 μL fill, Capiject, Terumo Medical Corporation, Elkton, MD) for sera lead analysis. From 2010 to 2012, we obtained approximately 2.5-mL blood sample using a 3-mL syringe and 25 gauge needle for a larger purple-top EDTA tube (1.3-mL fill, Sarsted, Inc., Newton, NC) and a green-top heparin tube (200-400 μL fill, Becton, Dickinson and Company, Franklin Lakes, NJ). We removed the needle from the syringe before transferring the blood to the collection tubes. We collected additional blood, starting in 2010, to perform manual complete blood counts (purple-top tube), sera chemistries, and venous blood gas (green-top tube) in addition to the lead study.

For lead analyses, we used the LeadCare I point-of-care analyzer (ESA Biosciences, Inc., Chelmsford, MA) with an upper detection limit of 65 μg/dL (3.1 μmol/L) and lower limit of 0 μg/dL (0 μmol/L). The environmental operating range for this system is 54°F-97°F (12°C-36°C) and 12%-80% humidity (User Guide, Blood Lead Testing System, p/n 70-2235, Rev. L, ESA Biosciences).

We used a Swift M7000 microscope (Swift Optical Instruments, Inc., San Jose, CA) to perform manual blood counts. This microscope has a rechargeable battery that, when fully charged, provides 50 hours of power in the field. We performed manual complete blood counts using an avian-specific total leukocyte counting system (Avian Leukopet, Vetlab Supply, Palmetto Bay, FL). We used a standard hemacytometer for total white blood counts. We air-dried blood smears on glass slides and stained them with a Dip Quick Stain (J-322, Jorgensen Laboratories, Inc., Loveland, CO). We interpreted cell morphology under an oil immersion lens. We performed blood counts on the day of collection and differentials 2-3 days later. In addition to differential counts, we also evaluated thrombocytes and erythrocytes on the glass slide blood smears.

In addition to hooding chicks to minimize the potential for a stress or physiological leukocytosis, we obtained blood samples at the onset of the restraint.[7,8] In 2010, we made blood smears using the samples in the EDTA tubes several hours after collection. We hypothesized that the anticoagulant and high environmental temperatures created artifacts, making cellular morphologic interpretation problematic. As such, we did not use any data from 2010. In 2011, we made 3 fresh blood smears on glass slides immediately after venipuncture with whole nonanticoagulated blood in the field. We found that the fresh smears created fewer artifacts making cellular identification and interpretation easier.

We determined packed cell volume (PCV) by centrifuging blood from the EDTA tubes in a capillary tube with a portable microcentrifuge (Zipocrit, LW Scientific, Inc., Diversified Instrument Service, Evansville, IN). We centrifuged the samples for 5 minutes at 11,000 rpm and used a card reader to estimate the PCV from the spun capillary tube. We determined the total protein (TP) from plasma using a portable clinical refractometer (J-351, Jorgensen Laboratories, Inc., Loveland, CO).

We determined sera chemistries, including the hematocrit (Hct), with an i-STAT portable clinical analyzer (Abaxis, Inc., Union City, CA). A study with domestic chickens concluded that the i-STAT (EG7+ cartridge) provided sufficient accuracy to use for critically ill avian patients.[9] On hot days, we kept the i-STAT in a freezer bag and used instant cold packs (instant ice compress, Duro-Med Industries, Waukegan, IL) to maintain the analyzer within its operating temperature range, 61°F-86°F (16ºC-30ºC). For blood chemistry analyses in 2011, we used the EC8+ cartridge (Hct, Hb, BUN, glucose, Cl^-, Na^+, K^+, pH, pCO_2, HCO_3, and TCO_2) and in 2012, we used the CG8+ cartridge (Hct, Hb, ionized calcium [iCa], glucose, Na^+, K^+, pH, pCO_2, HCO_3, TCO_2, base excess, pO_2, and sO_2). For blood gas analyses, we used venous blood and the CG4+ cartridge (pH, pCO_2, pO_2, base excess, HCO_3, TCO_2, sO_2, and lactate). We used the i-STAT to calculate the hemoglobin, HCO_3, TCO_2, sO_2, base excess, and anion gap analyte values.

We collected fecal material for analysis, only if it appeared to be fresh. Because of the high environmental temperature and low humidity in IN, avian feces desiccate quickly, rendering the sample useless. When we collected fresh feces, we analyzed the sample later that day using both a direct and a sugar flotation technique. For floatation, we used the Sheather sugar flotation technique.[10] We mixed the fecal sample with 20 mL of the sugar solution and then poured the solution into a 15-mL test tube until a meniscus is formed. We placed a cover slip on top of the meniscus and allowed it to sit for 60 minutes before reading at 10× and 40×.