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FROM THE FIELD

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California condor poisoned by lead, not copper, when both are ingested: A case study

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Abstract

Lead poisoning from feeding on carcasses shot with lead-based ammunition is a well-known threat to wildlife. Thus, nonlead (e.g., copper-based) ammunition is promoted as a safe alternative. We present a unique situation of a male California condor (*Gymnogyps californianus*) discovered with both a lead fragment and a copper bullet in his digestive tract simultaneously. We show that ingestion of a copper bullet did not result in elevated blood copper concentrations, while ingestion of a lead fragment contributed to lead toxicity. Our findings can inform nonlead ammunition outreach efforts by demonstrating that ingestion of a copper-based bullet did not result in the poisoning of a California condor.

KEYWORDS

ammunition, California condor, case study, copper, *Gymnogyps californianus*, lead poisoning

Lead exposure from ingestion of spent lead-based ammunition is a threat to wildlife globally (Fisher et al. 2006, Haig et al. 2014, Pain et al. 2019). Nonlead (e.g., copper-based) ammunition is recognized as an effective alternative for hunters and shooters (Gremse et al. 2014, Schlichting et al. 2017) and promoted to reduce preventable lead exposure to humans and scavenging wildlife (e.g., Camenzind 2019). In addition, in California (USA), legislative restrictions on the use of lead-based ammunition (State of California 2007, State of California 2013) have likely resulted in the expanded use of nonlead ammunition throughout the state. However, some have expressed concern about copper toxicity to nontarget wildlife and the potential for future restrictions on the use of copper-based ammunition (D. Ryan, personal observation, Pinnacles National Park). Here we present a case study of a unique situation with respect to a wild California condor (*Gymnogyps californianus*) that ingested both a lead fragment and a copper bullet to illustrate the risks of lead and copper toxicity from these ammunition types.

STUDY AREA

California condor studbook number 1034 fledged from a wild nest in the area surrounding Pinnacles National Park, Paicines, CA, USA and was part of the central California flock whose range generally occupies the region from San Luis Obispo County northward to Santa Clara County, California, USA.

METHODS

California condor 1034 case study

California condor studbook number 1034 is a male condor hatched in the wild on 28 April 2020 and managed by Pinnacles National Park. On 31 March 2021, during a routine health check, condor 1034 had an elevated field blood lead test (high reading [$\geq$65 µg/dL] on a LeadCare II Analyzer [Meridian Bioscience, Cincinnati, OH, USA]) and was transferred to a medical facility for treatment. A radiograph revealed several bright opacities suspected to be metal fragments and one large opacity suspected to be a bullet within the ventriculus (Figure 1A). One fragment (subsequently identified as lead-based) and the bullet (subsequently identified as copper-based) were collected after regurgitation for analysis (Figure 1B). See Appendix A for detailed clinical treatment timeline and Appendix B for California condor casting therapy technique.

Blood collection

Blood sampling for condor 1034 (lead and copper analysis) and 28 other condors (copper analysis) was part of routine and permitted semi-annual health checks on California condors conducted between 2007 and 2021 as part of ongoing endangered species management efforts under the authority of the U.S. Fish and Wildlife Service and with approval from the National Park Service Institutional Animal Care and Use Committee. Briefly, biologists captured free-flying condors using double-door traps attached to flight pens. Condors were netted and then restrained by hand for health examinations, including blood sampling, as well as replacement of transmitters if needed. Whole blood samples (1–3 mL) were collected from the metatarsal vein and placed into low-lead Vacutainers (Fisher Scientific, Pittsburgh, PA, USA) as previously described (Finkelstein et al. 2012). Blood samples were analyzed at the University of California Santa Cruz (UCSC) with approval from the UCSC Animal Care and Use Committee.

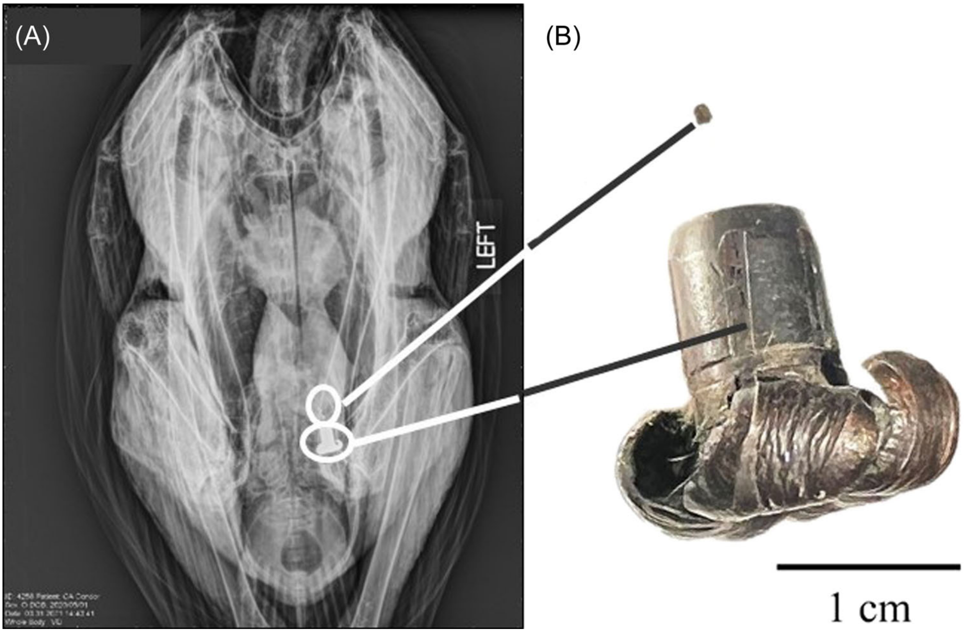

FIGURE 1 Metallic objects observed by radiograph and recovered from condor 1034's digestive tract. A) Condor 1034's ventrodorsal radiograph shows ingested spent ammunition in lower digestive tract. The small radiopaque objects circled above in white are suspected lead ammunition fragments. Circled in white below is the bullet identified as copper ammunition. B) Spent bullet identified as copper (below) compared to a fragment identified as lead (smaller item above); both items were photographed after recovery from the digestive tract of Condor 1034.

Fragment processing

Radiographed, opacity-positive casting material from condor 1034 was dissected, and a metallic fragment was collected. Although the radiograph showed the intact bullet and multiple small, bright opacities (Figure 1A), only one small metallic fragment was recovered from the casting material. The recovered fragment and bullet were individually cleaned and leached in 1 mL 1% HNO_3 for ~30 seconds, as previously described (Finkelstein et al. 2012). Leachates were screened for lead and copper by optical emission spectrometry (OES, Perkin-Elmer Optima 4300 DV). Analytical limits of quantitation (LOQ, reported here as 10x the standard deviation of three blank samples) were 0.096 µg/mL for lead and 0.002 µg/mL for copper. The lead-positive fragment was diluted and further analyzed for lead isotopic composition as described below.

Blood sample processing

Blood samples were processed using established trace metal clean techniques (Finkelstein et al. 2012). Briefly, blood was digested overnight with 2 mL of sub-boiling concentrated HNO_3 in closed Teflon vials. The concentrated acid was evaporated to dryness and samples were reconstituted in 1% HNO_3 for analysis. In addition to condor 1034's blood sample, archived digested blood samples collected between 2007 and 2021 from condors in California with a range of blood lead values (1.3–550 µg/dL, n = 28) and no known copper exposure (e.g., no observed copper bullets for radiographed individuals) were analyzed for copper concentrations.

Lead and copper analysis

Lead and copper concentrations and lead isotopic compositions were determined by inductively coupled plasma mass spectrometry (ICP-MS; Thermo Element XR high-resolution, Waltham, MA, USA) at the UC Santa Cruz Plasma Analytical Laboratory (Research Resource Identification, RRID:SCR_021925). Lead concentrations and isotopic compositions were determined by measuring masses of ^{206}Pb, ^{207}Pb, and ^{208}Pb as previously described (Finkelstein et al. 2010, 2012). Thalium-205 was used as an internal standard. The precision (2x the relative standard deviation or 2 RSD) of lead isotope ratios was ~0.10% based on condor tissue samples ($n = 4$) analyzed within an analytical run. Between-run (i.e., long-term over several years) measurement precision was <0.20% (2 RSD) based on repeated measurements of blood leachate samples. Isotope ratios (^{207}Pb/^{206}Pb) that differed by <0.20% (i.e., 2 RSD of long-term measurement precision) were considered measurably indistinguishable. Lead recoveries using Standard Reference Material NIST 955 C (bovine blood, level 4) was 95 and 96% ($n = 2$). Copper concentrations were determined by measuring the mass of ^{63}Cu. Rhodium-103 was used as an internal standard. Copper recoveries using Standard Reference Material NIST 2670a (freeze-dried urine, high level) ranged from 82–85% ($n = 3$).

RESULTS

The bullet from condor 1034's ventriculus was identified as a commercially available copper round while the recovered fragment was lead-based

Upon visual examination, the recovered bullet was identified as Federal Ammunition brand Trophy Copper bullet style, a nonlead round designed for big game. Using optical emission spectroscopy on leachates (~30 seconds in weak acid), the bullet leachate was positive for copper (~650 μg total copper leached) and below detection for lead while the leachate of the fragment recovered from the casting was positive for lead (~21 μg total lead leached) with a low amount of copper (~2.5 μg total copper leached).

Condor 1034 had elevated whole blood lead but not whole blood copper values

Condor 1034's whole blood copper concentration was 38.9 μg/dL when chelation treatment was initiated to treat lead poisoning (blood lead 82.9 μg/dL). Compared to a representative sample of other free-flying condors, condor 1034's blood copper level was in the 86th percentile and close to the median value of 33.1 μg/dL ($\bar{x} = 32.0 \pm 9.78$ SD μg/dL, $n = 28$; Figure 2). Condor 1034's whole blood copper concentration was also similar to mean whole blood copper concentrations reported for northern goshawks (39.9 ± 7.3 SD μg/dL [Stout et al. 2009]) and Griffon vultures (20.39 ± 5.92 SD μg/dL [Espín et al. 2014]). We found no relationship between whole blood concentrations of copper and lead (Spearman's $\rho = -0.224$, $P = 0.253$, $n = 29$; Figure S1, available in Supporting Information) illustrating a lack of association between copper and lead exposure in free-flying condors.

Lead isotopic compositions indicate that the recovered lead fragment was the source of lead poisoning to condor 1034

The ^{207}Pb/^{206}Pb ratio of condor 1034's whole blood was measurably indistinguishable from the ^{207}Pb/^{206}Pb ratio of the recovered lead fragment (blood ^{207}Pb/^{206}Pb = 0.8365 versus fragment ^{207}Pb/^{206}Pb = 0.8389; Figure S2, available in Supporting Information) strongly indicating that the lead fragment was the source—or one of the sources—of lead poisoning in condor 1034.

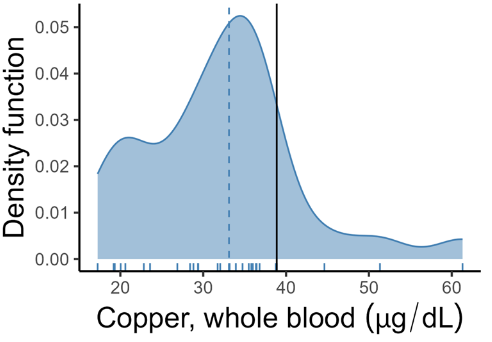

FIGURE 2 Kernel density function for whole blood copper concentrations (µg/dL) measured in free-flying California condors. The median copper concentration (dotted blue line) was 33.1 µg/dL (min–max = 17.3–61.3 µg/dL, n = 29). Condor 1034 (solid black line) had a copper concentration of 38.9 µg/dL. Based on bootstrapped samples (n = 100,000), condor 1034's copper concentration was at the 86th percentile of the observed distribution. Individual blood copper values shown on x-axis.

DISCUSSION

Accidental ingestion of lead-based ammunition residues from feeding on terrestrial carcasses is the principle source of lead poisoning for California condors (Finkelstein et al. 2012). Although California has implemented a ban on using lead ammunition for hunting (State of California 2013), condors continue to be lead poisoned on a regular basis (U.S. Fish and Wildlife Service 2021). Condor 1034's blood lead level was ~2-fold higher than Pinnacles National Park's threshold level for treatment for lead poisoning (Welch et al. 2022) and lead isotopic compositions of condor 1034's blood and the recovered fragment indicated that the small lead fragment was a source of his lead poisoning. Although condor 1034 had multiple small lead ammunition fragments that might have contributed to his elevated blood lead level, these fragments were considerably smaller than the ingested intact copper bullet. The occurrence of an intact bullet along with small fragments of lead in condor 1034's digestive tract is unsurprising as copper-based bullets tend to retain their mass upon impact, unlike lead-based bullets which fragment into numerous small pieces (Stroud and Hunt 2009, Menozzi et al. 2019).

We acknowledge that with a sample size of one, such as reported here for condor 1034 and for case studies in general, caution is required when interpreting the importance of the findings. We also note that the timing of condor 1034's ingestion of the copper bullet and lead fragments is not known. However, condor 1034 had the copper bullet in his digestive tract for at least 20 days with no increase in serum copper levels (49 µg/dL when admitted to the Oakland Zoo and 42 µg/dL 20 days later when transferred to the Los Angeles Zoo [Appendix A]), further supporting the conclusion that ingestion of the copper bullet did not result in elevated copper levels. Experimental dosing studies have shown that ingested copper pellets do not result in copper toxicity in other avian species (Christian Franson et al. 2012, Krone et al. 2019) and our case study is consistent with these findings. Nonetheless, we recommend further research that builds upon the copper levels reported here and elsewhere (e.g., Griffon vultures; Espín et al. 2014) to establish baseline levels in avian scavengers potentially at risk of ingestion of copper-based ammunition.

CONSERVATION IMPLICATIONS

Lead-based ammunition is a well-known threat to human and wildlife health (Bellinger et al. 2013). The use of nonlead alternatives, such as copper-based bullets, has been proposed and promoted (Cade 2007, Thomas 2013). However, facilitators that conduct outreach on nonlead ammunition have encountered concerns about the potential toxicity of ingested copper ammunition and the possible implementation of regulations on copper ammunition as a result (D. Ryan, personal observation, Pinnacles National Park). The case study of California condor 1034 demonstrates that i) ingestion of a spent copper bullet did not result in elevated blood copper concentrations in an avian scavenger, consistent with published studies showing extremely low risk of copper toxicity from ingestion of copper-based items, and ii) ingestion of a small lead fragment did contribute to lead toxicity. Illustrative case studies such as the one reported here can bolster ongoing efforts to educate the general public about the benefits of nonlead ammunition (e.g., North American Non-lead Partnership) by confirming the safety of copper-based ammunition when ingested by wildlife.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

ETHICS STATEMENT

California condor samples were collected by staff affiliated with California condor recovery at Pinnacles National Park, Oakland Zoo, and Los Angeles Zoo and Botanical Gardens as part of ongoing management efforts permitted by the U.S. Fish and Wildlife Service (USFWS permit numbers ES157291-3 and ES026659-16, National Park Service IACUC protocol CA_PINN_Welch_Condor_2021.A3). Samples were received and processed by UCSC under the Vertebrate Sample Use Protocol Finkm2001.

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SUPPORTING INFORMATION

Additional supporting material may be found in the online version of this article at the publisher's website.

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APPENDIX A: CONDOR 1034 CLINICAL TREATMENT TIMELINE

26 March 2021

Condor 1034 was captured at Pinnacles National Park as part of scheduled semi-annual monitoring of Condors in California.

31 March 2021

A field blood lead test administered by Pinnacles National Park condor field biologists on condor 1034 resulted in high reading (≥65 μg/dL) on a LeadCare II Analyzer (Meridian Bioscience) during a routine health check. Condor 1034 was transferred to the Oakland Zoo Veterinary Hospital (California, USA) for treatment for elevated lead exposure. No abnormalities were noted on physical exam. Radiographs, both lateral and ventrodorsal views, showed multiple small, bright opacities interpreted as metal fragments and one large opacity suspected to be a copper bullet within the ventriculus. Condor 1034 was treated with lead chelation and casting protocols (Appendix B) to reduce blood lead levels and facilitate regurgitation of the suspected bullet and metallic fragments. Condor 1034's serum copper level was 49 µg/dL (California Animal Health & Food Safety Laboratory, Davis, USA).

12 April 2021

Condor 1034 remained bright and alert, with no clinical signs of copper or lead toxicity. Radiographs confirmed that the small metallic fragments were cleared with casting material via regurgitation, but the suspected copper bullet remained in the ventriculus.

14 April 2021

Condor 1034 was transferred to Los Angeles Zoo (LAZ; California, USA) for additional attempts at retrieval of the suspected copper bullet from the ventriculus to confirm its composition.

19 April 2021

Condor 1034 regurgitated the bullet (Appendix B) and continued to show no clinical signs of lead or copper toxicity while at LAZ. Condor 1034's serum copper concentration was 42 µg/dL (California Animal Health & Food Safety Laboratory).

28 April 2021

Condor 1034 was released back into the wild at Pinnacles National Park, California, USA.

APPENDIX B: CALIFORNIA CONDOR CASTING THERAPY TECHNIQUE

The casting therapy technique to collect metallic densities visible via radiograph in the ventriculus of California condors without surgery was developed by Los Angeles Zoo condor keeper Michael Clark. During casting therapy ~100–200 g of food including mammal skin (20–40% of what condors can process in one day) is offered. This food can pass through the ventriculus within about 12 hours and by 24 hours most of the food has passed through the entire digestive tract, which leaves a mass of indigestible material, consisting largely of hair, collected in the ventriculus. Following treatment, the bird will often cast up a pellet on its own without provocation and the vast majority of birds cast within 23 hours. Any indigestible debris, such as dirt, pebbles, vegetation, or metallic densities, remaining in the ventriculus is usually bound by the hairy casting material and expelled from the ventriculus with the act of casting. The detailed protocol consists of these 3 steps:

i) Keepers force feed the restrained patient strips of rat or rabbit skin containing hair soaked in warm blood or warm bloody water. It is important that enough hair is offered to form a sizeable casting.
ii) Keepers immediately offer strips of fresh warm bloody horsemeat or other enticing food to reduce the likelihood of immediate regurgitation of force-fed casting material. To further reduce the bird's urge to quickly regurgitate, it is held without disturbance for 5–10 minutes to allow it to acclimate to the feeling of its crop contents.
iii) If after ~24 hours the bird has not cast up a pellet, casting is encouraged by placing an enticing food item outside the enclosure where the bird can see it but cannot access it. Since condors and raptors sometimes cast when they know they are about to eat, this visual presence of food can prompt them to cast.