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Serum and Plasma Cholinesterase Activity in the Cape Griffon Vulture (*Gyps coprotheres*)

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ABSTRACT: Vulture (Accipitridae) poisonings are a concern in South Africa, with hundreds of birds dying annually. Although some of these poisonings are accidental, there has been an increase in the number of intentional baiting of poached rhinoceros (Rhinocerotidae) and elephant (Elephantidae) carcasses to kill vultures that alert officials to poaching sites by circling overhead. The primary chemicals implicated are the organophosphorous and carbamate compounds. Although most poisoning events can be identified by dead vultures surrounding the scavenged carcass, weak birds are occasionally found and brought to rehabilitation centers for treatment. The treating veterinarian needs to make an informed decision on the cause of illness or poisoning prior to treatment. We established the reference interval for serum and plasma cholinesterase activity in the Cape Griffon Vulture (*Gyps coprotheres*) as 591.58–1,528.26 U/L, providing a clinical assay for determining potential exposure to cholinesterase-depressing pesticides. Both manual and automated samplers were used with the butyrylthiocholine method. Species reference intervals for both serum and plasma cholinesterase showed good correlation and manual and automated measurements yielded similar results.

Key words: Cape Griffon Vulture, organophosphate toxicity, reference interval, serum/plasma cholinesterase.

The Old World vultures (Accipitridae) are large, obligate-scavenging, gregarious birds, of which nine species occur in southern Africa (Mundy et al. 1992). Under natural conditions, vultures are reliant on predator kills of wild ungulates for food. When predator kills are not available, the birds will feed on any carrion, including farm mortalities. Irrespective of the source of their food, when food is available it is not unusual to see birds circling or feeding on these carcasses. Because of their gregarious nature and group feeding behavior, only 0.1–0.8% of available carcasses need to contain lethal compounds to cause mass mortality as represented in the 99% population decline in Indian Vultures (*Gyps indicus*) exposed to diclofenac in India (Green et al. 2004).

In the southern African context, where diclofenac is not available, vultures face other chemical threats (Ogada 2014). In the past, this typically resulted from the baiting of carcasses with pesticides for the control of problem predators such as jackals (*Canis* spp.). More recently, poachers also use poison bait to kill vultures that quickly begin circling poached rhinoceros (Rhinocerotidae) and elephant (Elephantidae) carcasses, a behavior that has been used by law enforcement officers to locate the carcasses and poachers. Although several poisons have been implicated, the most common agents are the organophosphorous compounds and the carbamate aldicarb (Ogada 2014). Although baiting of carcasses generally results in massive die-offs, occasionally birds are presented for veterinary treatment. Clinical signs of intoxication are generally nonspecific, making effective treatment difficult (Naidoo et al. 2011). Although plasma/serum cholinesterase (ChE) activities can be used as evidence to support poisoning by organophosphate and carbamate compounds (Fossi et al. 1992), the species reference intervals for many southern African vulture species are undetermined.

The ChE enzyme is found throughout the body of vertebrates, with the highest levels in liver, pancreas, heart, serum, and brain. Therein it modulates normal organ function or plays a role in drug metabolism by terminating the function of the neurotransmitter acetylcholine. Brain ChE activity has typically been the assay used to establish diagnostic standards and determine poisoning due to

ChE-inhibiting compounds postmortem; however, ChE activity can also be quantified using serum or plasma under clinical settings. Although in mammals the ChE is found within erythrocytes and plasma, in birds the enzyme is found within plasma and serum (Roy et al. 2005). We estimated the plasma and serum activity of ChE in adult Cape Griffon Vulture (*Gyps coprotheres*), one of southern Africa's endangered vulture species.

For the manual quantification of ChE activity, 17 healthy adult Cape Griffon Vultures, housed at the Vulture Programme (VulPro, Ritfontein, South Africa) without known exposure to ChE-inhibiting compounds for >1 yr were sampled in 2010. These previously wild birds were in captivity due to physical injuries that precluded their release. Blood (2 mL) was collected in a 5-mL syringe precoated with ethylenediaminetetraacetic acid (EDTA) in water for plasma samples or into a nontreated syringe for serum prior to being transferred into 5-mL evacuated serum tubes (BD Vacutainer®, Johannesburg, South Africa). Both the EDTA-coated and noncoated sample tubes were centrifuged at 2,500 × G (Beckman Coulter, Johannesburg, South Africa) for 10 min, stored at 3 C, and analyzed within 3 d of collection. For the determination of ChE activity, samples were analyzed manually using the reagents supplied in the commercial analytical kit (Cholinesterase Gen.2, Roche, Johannesburg, South Africa), consisting of R1 (pyrophosphate buffer 92 mmol/L [pH 7.7], potassium hexacyanoferrate 2.4 mmol/L) and R2 (Good's buffer 10 mmol/L [pH 4.0], butyrylthiocholine 46 mmol/L). A reagent blank was prepared by reacting 125 mL of R1 (preincubated at 37 C for 5 min) with 10 µl of R2 for 90 s and subsequent reading at 410 nM on a Versamax 96-well microplate reader (Molecular Devices, Sunnyvale, California, USA) with a corrected 1-cm path length. For the enzyme activity, 125 µL of R1 was mixed with 3 µL of serum/plasma and incubated for 5 min in a water bath at 37 C. The samples were transferred to 96-well microtiter plates and mixed with 10 µL of R2. Absorbance was measured at 410 nm, 90 s after adding R2. Enzyme activity (b) was expressed in international enzyme units per liter (U/L) at 37 C and calculated by $b=aV/\varepsilon lv$, where a is the change in absorbance, V is the total reaction volume (138 µL), ε is the molar absorption coefficient potassium hexacyanoferrate at 410 nm (96.1 m^2/mol), l is the path length (0.01 m), and v is the serum/plasma volume (3 µL) (Furlanello et al. 2006).

For the automated serum estimation of ChE activity, 25 additional adult Cape Griffon Vultures (unknown sex) without known exposure to ChE-inhibiting compounds had blood collected into evacuated serum tubes (5-mL BD Vacutainer) from the tarsal vein. Serum samples were submitted to a commercial clinical pathology laboratory (Ampath, Pretoria, South Africa) for analysis on a Roche automated clinical chemical analyzer using the Cholinesterase Gen.2 analyzer kit, using the method described earlier.

The Shapiro-Wilk test of normality was performed on nontransformed serum and plasma ChE activity values. Because the automated ChE data were not normally distributed, activity values were transformed (natural logarithm [Ln]). The reference intervals were calculated as mean±2×SD, except for automated parameter that required natural logarithmic transformation. For the latter the reference interval was calculated as the antilog of the LnMean±2×LnSD (Bland and Altman 1996). Results are also presented as descriptive statistics with 95% confidence intervals.

The results obtained from manual analysis are presented in Table 1. The EDTA and serum samples had mean ChE activity of 1,070.59±171.53 and 1,140.34±172.80 U/L respectively. The ChE reference intervals were 727.53–1,413.64 U/L and 794.74–1,485.94 U/L for the plasma and serum samples respectively. No significant difference was found between serum and plasma (P=0.12; Student's t-test), showing that either sample would be adequate for determination of ChE activity. This was in agreement with the recommendation of the manufacturer that EDTA- or heparin-treated blood (plasma) or serum samples from mammals could be used for analysis. The automated samples yielded a mean ChE activity of 976.19±1.28 U/L and a wider, albeit similar, reference interval of

TABLE 1. Descriptive statistics for cholinesterase (ChE) activity (U/L) in Cape Griffon Vultures (*Gyps coprotheres*) from South Africa, measured using manual and automated samplers in plasma and serum, 2010.

Statistics	Manual		Automated		Combined serum
	Plasma	Serum	LnChE[a]	ChE[b]	
Number of samples	17	17	25	25	42
Mean	1,070.59	1,140.34	6.88	976.19	1,059.92
95% Lower confidence interval	982.40	1,051.50	6.76	859.86	986.95
95% Upper confidence interval	1,158.78	1,229.19	7.01	1,108.25	1,132.90
Standard deviation	171.53	172.80	0.25	1.28	234.17
Minimum	886.73	724.65	6.50	662.00	662.00
Maximum	688.32	780.06	7.46	1,732.00	1,732.00
Lower reference interval	727.53	794.75	6.39	595.91	591.58
Upper reference interval	1,413.64	1,485.94	7.38	1,599.13	1,528.26

[a] Data were natural-log (Ln) transformed to achieve a normal distribution for statistical analysis.
[b] Represents the antilog values following data evaluation using descriptive statistics.

595.91–1,599.13 U/L. When the data for the serum ChE activity were pooled, the reference interval was 591.58–1,528.26 U/L. Based on the larger sample size used for the calculation of the latter value, this is most likely the true species reference interval for serum activity.

Although the serum/plasma ChE activity was lower than that generally seen for mammals, the reference intervals we determined were similar to those for other vultures, including Lammergeier (*Gypaetus barbatus*) adults (688±195.1 U/L; Hernandez and Margalida 2010), and the Egyptian Vulture (*Neophron percnopterus*) (709±192 U/L; Roy et al. 2005). Activities were also similar to those for Red-Tailed Hawks (*Buteo jamaicensis*) (790±162 U/L; Hooper et al. 1989). This finding also corresponded with published literature showing that the serum/plasma ChE activity of raptors is generally lower than that of omnivorous and herbivorous bird species. Serum/plasma ChE activity was, however, lower than that reported for juvenile (±40-d-old) African White-backed Vultures (*Gyps africanus*) at 1,796±158.33 U/L (van Wyk et al. 1998). The reason for the 50% lower value in the Cape Griffon Vulture is unknown, especially because previous studies have indicated that these two vulture species are closely related. One possible reason could be the use of different markers for the colorimetric quantification. Van Wyk et al. (1998) used the acetylthiocholine reaction, whereas we used the butyrylthiocholine method because it is readily available in South Africa. The results from the other bird species with similar activities were achieved using either the acetylthiocholine method (Hooper et al 1989; Roy et al 2005) or the butyrylthiocholine method (Hernandez and Margalida 2010). Another possible reason could be age-related declines in plasma/serum ChE activity; a similar trend was reported for the Peregrine Falcon (*Falco peregrinus*; del Pilar Lanzarot et al 2001). Finally, a major interspecific difference is possible.

In conclusion, Cape Griffon Vulture serum/plasma ChE activities were similar to those of other raptors, with a serum reference interval of 591.58–1,528.26 U/L. Both manual and automated quantification of serum/plasma ChE activity provide reliable reference ranges for the Cape Griffon Vulture.

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LITERATURE CITED

Bland JM, Altman DG. 1996. Transformation, mean and confidence intervals. *Brit Med J* 312:1079.

del Pilar Lanzarot M, Montesinos A, San Andrés MI, Rodríguez C, Barahona MV. 2001. Hematological, protein electrophoresis and cholinesterase values of

free-living nestling Peregrine Falcons in Spain. *J Wildl Dis* 37:172–178.

Fossi MC, Leonzio C, Massi A, Lari L, Casini S. 1992. Serum esterases inhibition in birds: A nondestructive biomarker to assess organophosphorus and carbamate contamination. *Arch Environ Contam Toxicol* 23:99–104.

Furlanello T, Simonato G, Caldin M, De Lorenzi D, Lubas G, Bernardini D, Solano-Gallego L. 2006. Validation of an automated spectrophotometric assay for determination of cholinesterase activity in canine serum. *Vet Res Commun* 30:723–733.

Green RE, Newton I, Shultz S, Cunningham AA, Gilbert M, Pain DJ, Prakash V. 2004. Diclofenac poisoning as a cause of vulture population declines across the Indian subcontinent. *J Appl Ecol* 41:793–800.

Hernandez M, Margalida A. 2010. Hematology and blood chemistry reference values and age-related changes in Bearded Vultures (*Gypaetus barbatus*). *J Wildl Dis* 46:390–400.

Hooper MJ, Detrich PJ, Weisskopf CP, Wilson BW. 1989. Organophosphorous insecticides exposure in hawks inhabiting orchards during winter dormant-spraying. *Bull Environ Contam Toxicol* 42:651–659.

Mundy P, Butchart D, Ledgar J, Piper S. 1992. *The vultures of Africa*. Acorn Books CC, Halfway House, South Africa, 464 pp.

Naidoo V, Wolter K, Espie I, Kotze A. 2011. Vulture rescue and rehabilitation in South Africa: An urban perspective. *J S Afr Vet Assoc* 82:24–31.

Ogada DL. 2014. The power of poison: Pesticide poisoning of Africa's wildlife. *Ann N Y Acad Sci* 1322:1–20.

Roy C, Grolleau G, Chamouland S, Riviere J. 2005. Plasma B-esterase activities in European raptors. *J Wildl Dis* 41:184–208.

van Wyk E, van der bank H, Verdoorn GH. 1998. Dynamics of haematology and blood chemistry in free-living African Whitebacked Vulture (*Pseudogyps africanus*) nestlings. *Comp Biochem Physiol A Mol Physiol* 120:495–508.

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