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# HEMATOLOGY AND BIOCHEMISTRY REFERENCE VALUES IN NESTLING RED KITES (*MILVUS MILVUS*) IN SHORT-TERM HUMAN CARE IN ENGLAND

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*Abstract:* Between the years 2022 and 2023, 62 red kite (*Milvus milvus*) nestlings were translocated from England to Spain to bolster declining populations in mainland Europe as part of a wider conservation initiative. Health examinations were undertaken by veterinarians ahead of translocation, including examination of hematology and biochemistry parameters from blood samples. This study aimed to establish reference values for these parameters in nestling red kites for use in future translocations or for other clinical purposes. All individuals included in the analysis were clinically healthy at the time of sampling. Biochemical reference intervals were comparable to published values for other Accipitridae, although differences in hematology were noted: PCV was generally lower; and WBC counts higher than (up to triple) those reported for related species of a similar age. It is hypothesized that these differences reflect species variations or the effects of the stress of recent capture on the immune system of the red kites. A *Leucocytozoon* species was identified on blood smears of six of the red kites. The reference intervals presented in this study are representative of free-living red kite nestlings in England that have recently been captured for conservation translocation purposes.

## INTRODUCTION

Red kite (*Milvus milvus*) populations have fluctuated throughout Europe in recent years; a reintroduction project in England, established in the late 1980s, successfully enabled substantial population growth and range expansion of the species that had been persecuted to extinction in England.[1,3,17] The red kite is currently listed as least concern on the International Union for the Conservation of Nature's Red List of Threatened Species, although populations across other parts of Europe have suffered drastic losses. Since 2011 in continental Spain, the red kite has been considered to be at risk of extinction due to anthropogenic threats.[11,16] In response, a translocation program of red kites from England was developed, and in the years 2022 and 2023, 62 nestling red kites were successfully translocated and released.

Assessment of hematology and serum biochemistry, using validated reference ranges, is an essential tool to aid in determining the health status of an individual animal. The aim of this study was to establish hematologic and biochemical reference values for nestling red kites and identify any unique, species-specific findings. To the authors' knowledge, this is the first publication to evaluate these indices in nestling red kites, and the information is likely to be useful in future, given the ongoing conservation interventions involving this species across the world.

## MATERIALS AND METHODS

The study was undertaken in accordance with American Society for Veterinary Clinical, Pathology Quality Assurance and Laboratory Guidelines.[9]

### Study population

Whole blood samples were collected from 62 nestling red kites in June 2022 and June 2023. Red kites between 4 and 6 wk of age were collected from monitored wild nests in Northamptonshire, United Kingdom. Between 1 to 4 d after capture, they were examined, including assessment of the cardiovascular, respiratory, and musculoskeletal system, a full ophthalmic examination, and examination of the oral cavity and cloaca. A microchip was placed in the left pectoral muscle, and venipuncture was performed. Whole blood samples were collected by licensed veterinarians for routine diagnostic purposes under the Veterinary Surgeons Act (1966),[20] as part of health examinations ahead of a conservation translocation to Spain. Prior to sampling, the nestling red kites had been housed in pairs (and one group of three) in short-term purpose-built pens. They had been fed frozen thawed,

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water-soaked rat or freshly trapped squirrel carcasses once daily.

Birds were manually captured and restrained, and approximately 1.2 ml of blood was collected from either the jugular or brachial vein and immediately placed into a tube containing lithium heparin. A fresh blood smear was made at the time of collection. On the same day as collection, blood samples and smears were transported to the Royal Veterinary College Diagnostic Laboratory (Hawkshead Campus, North Mymms, Hertfordshire, AL9 7TA, United Kingdom) for analysis. In June 2022, hematologic analyses were performed on all 30 birds, and biochemistry for 15 birds. Biochemistry was undertaken within 24 h for three of the blood samples. Twelve additional samples were stored at −80°C for 9 mon, at which point they were defrosted at approximately 5–7°C and transported to the same laboratory for biochemical analyses. In June 2023, all testing was performed within 24 h of sample collection. Red kites were DNA sexed through PCR on blood samples (AndDNA Laboratorio de Analisis Molecular, 14300 Cordoba, Spain) prior to release in Spain.

### Laboratory analyses

Reported parameters can be found in Tables 1 and 2. For WBC estimates, leukocytes were counted manually using a Neubauer hemocytometer (Hawksley & Sons Ltd, Lancing, Sussex BN15 8TN, United Kingdom) and using phase contrast microscopy. Counts were performed in 10 fields (40× objective, i.e., 400× magnification), and the mean was multiplied by 1.6 to calculate numbers in 10e9/L. Heterophil, lymphocyte, eosinophil, monocyte, and basophil values were obtained by undertaking a 100-differential count and multiplying the percentages with the estimated WBC. For PCV, capillary tubes were filled with the anticoagulated blood sample and sealed with clay. The tubes were centrifuged at 13000 g for 5 min via a microhematocrit centrifuge (Hawksley Haematospin 1400, Hawksley & Sons Ltd). The results were read manually with a Hawksley Micro Haematocrit Reader. All biochemical parameters were measured using an AU680 Clinical Chemistry Analyzer (Beckman Coulter, Brea, CA 92821, USA).

Blood smears were fixed and stained with a modified Wright stain and manually examined using a light microscope under 100× magnification by a clinical pathologist. The presence of hemoparasites was judged as low, medium, or high by the clinical pathologist on the basis of the subjective number of macro- and microgametocytes visualized through microscopic examination of blood cells.

**Table 1.** Results of data analyses for hematologic parameters from 58 red kite (*Milvus milvus*) nestlings. The table presents the reference interval, mean, and SD of the data for normally distributed data or median (25th, 75th) for abnormally distributed data, as well as the data distribution (Gaussian [G] or non-Gaussian [NG]) and statistical method used for each individual parameter.

| Parameter | n | Mean | Median | Lower reference limit (95% confidence interval) | Upper reference limit (95% confidence interval) | Distribution | Analysis method |
|---|---|---|---|---|---|---|---|
| WBC (10e9/L) | 58 | — | 25 (20.25, 30) | 13.48 (12.48–13.95) | 52.1 (50.2–59.9) | NG | N |
| Heterophils (10e9/L) | 58 | — | 11.76 (9.17, 15.32) | 5.57 (4.53–5.82) | 30.05 (26.59–38.53) | NG | N |
| Heterophils (%) | 57 | 49.19 ± 10.09 | — | 30.2 (26.63–33.68) | 67.24 (63.72–70.77) | G | P |
| Lymphocytes (10e9/L) | 58 | — | 5.59 (4.01, 6.79) | 2.61 (2.42–2.69) | 13.32 (11.65–16.51) | NG | N |
| Lymphocytes (%) | 58 | 23.28 ± 7.19 | — | 9.18 (6.53–11.84) | 37.37 (34.72–40.02) | G | P |
| Monocytes (10e9/L) | 56 | — | 1.35 (1, 1.79) | 0.55 (0.47–0.6) | 3.85 (3.69–4.55) | NG | N |
| Monocytes (%) | 57 | — | 5.0 (4, 8) | 2 (1.1–2) | 13 (13–14.8) | NG | N |
| Eosinophils (10e9/L) | 58 | — | 4.54 (2.81, 5.93) | 1.5 (1.29–1.54) | 18.95 (18.91–26.21) | NG | N |
| Eosinophils (%) | 58 | — | 18.5 (13, 23) | 6.48 (3.95–6.95) | 38.53 (38.05–41.95) | NG | N |
| Basophils (10e9/L) | 58 | — | 0.38 (0.2, 0.79] | 0 | 2.09 (1.79–2.92) | NG | N |
| Basophils (%) | 58 | — | 2.0 (1, 3) | 0 | 7.43 (7.05–9.53) | NG | N |
| PCV | 57 | — | 32 (29, 33) | 26.45 (25.45–26.9) | 36.0 (36–38) | NG | N |

Analysis method: N = non-parametric, P = Parametric.

**Table 2.** Results of data analyses for plasma biochemical parameters from 43 red kite (*Milvus milvus*) nestlings. The table presents the reference interval, mean and SD of the data, as well as the data distribution (Gaussian [G] or non-Gaussian [NG]) and statistical method used for each individual parameter.

| Parameter | n | Mean ± SD | Median (25th, 75th) | Lower reference limit (95% confidence interval) | Upper reference limit (95% confidence interval) | Distribution | Analysis method |
|---|---|---|---|---|---|---|---|
| Uric acid (μmol/L) | 42 | — | 306 (264, 355) | 166.15 (128.23–166.3) | 536.18 (535.35–556.78) | NG | N |
| Creatine kinase (U/L) | 42 | — | 1245 (1,019.5, 1,423.5) | 848.68 (811.35–849.35) | 2441.25 (2431.5–2819.12) | NG | N |
| Glutamate dehydrogenase (U/L) | 43 | — | 2.3 (1.55, 2.8) | 1.11 (1–1.12) | 6.64 (6.58–7.86) | NG | N |
| Total protein (g/L) | 42 | — | 30.6 (29.8, 33.5) | 26.24 (24.76–26.28) | 35.59 (35.57–35.95) | NG | N |
| Calcium (mmol/L) | 43 | 2.6 ± 0.1 | — | 2.41 (2.36–2.45) | 2.8 (2.76–2.84) | G | P |
| Inorganic phosphorus (mmol/L) | 43 | 2.04 ± 0.18 | — | 1.68 (1.6–1.76) | 2.4 (2.32–2.48) | G | P |
| Glucose (mmol/L) | 43 | 14.34 ± 1.64 | — | 11.13 (10.42–11.83) | 17.56 (16.86–18.27) | G | P |
| Bile acids (μmol/L) | 40 | — | 9.8 (6, 11.8) | 2.21 (1.56–2.22) | 20.39 (20.37–22.47) | NG | N |
| Aspartate aminotransferase (U/L) | 42 | 313.44 ± 65.36 | — | 192.5 (165.08–219.91) | 440.27 (412.84–467.68) | G | P |

Analysis method: N = non-parametric, P = Parametric.

### Statistical analyses

All statistical analyses were undertaken in R statistical software (version 4.2.2; R Core Team, Vienna, Austria). Reference intervals were attained using the package *referenceIntervals* (version 1.2.0).[8] Histograms were visually assessed alongside results of a Shapiro-Wilk test to determine the normality of each parameter. Parameters were studied, and outliers identified on the basis of clinical reasoning alongside the Dixon range statistic;[6] individual red kites for which more than two outliers were obtained across parameters were excluded from analyses. Any further outliers identified within each dataset were removed from individual parameter analysis only.

For parameters that showed a Gaussian distribution, the parametric method was used to attain 95% reference interval (RI). For parameters with a non-Gaussian distribution, the nonparametric method was used to attain a 95% RI. The 90% confidence intervals of the lower and upper reference limits were calculated using a bootstrap method.

## RESULTS

Blood samples were collected from 37 female and 2 male nestling red kites. Sixty-one birds were assessed as clinically healthy at the time of examination based on normal clinical examination. One red kite was excluded from the study population based on clinical suspicion of aspergillosis. The mean body weight of the birds at the time of sampling was 862.7 g (range 534 to 1,080 g). Three additional red kites were excluded from analyses due to each having more than two outlying results across the dataset. On examination of blood smears, six red kites were noted to have micro- and macrogametes present within red blood cells, consistent with *Leucocytozoon* sp. infection. Despite infection, five of these birds were included in analysis (the sixth red kite had already been excluded due to outlying results).

After outlier removal, hematology parameters from 58 nestling red kites were analyzed; one PCV value was missing, so 57 samples were included in this dataset. Biochemistry parameters from 43 red kites were analyzed. Results of these analyses, including distribution and statistical methods, are presented in Tables 1 and 2.

## DISCUSSION

This study presents the first published RIs for hematologic and biochemical blood parameters in nestling red kites. It is not possible to directly extrapolate these RIs to red kites of other ages, given the age-related differences reported in other raptor species.[7,10,13] However, they may provide a

useful basis for other age groups given the limited literature available for this species.

Results of biochemical parameters in red kites were generally comparable to other published work involving free-living Accipitridae nestlings,[12,14] although the mean bile acid concentration was approximately half that reported for nestling golden eagles (*Aquila chrysaetos*) and three times lower than nestling red kites in Germany. Within the current study, 15 blood samples used for biochemical analyses were stored for 7 mon at −80°C. Results from these 15 samples were comparable to the results from those samples analyzed within 24 h of collection, and studies indicate that biochemical parameters are stable in samples frozen at −80°C for up to 13 mon.[2,5,18] However, no avian-specific literature on the effects of storage is available, and it is possible storage led to falsely depleted or elevated levels of some parameters, reducing the reliability of these results.

Hematology RIs are published for the closely related black kite (*Milvus migrans*), as well as for brahminy (*Haliastur indus*) and black-shouldered (*Elanus caeruleus*) kites, and other members of the Accipitridae family. Moreover, some hematologic values are published for a group of 29 nestling red kites in Germany, although RIs were not established statistically.[19] The median PCV was lower in the nestling red kites in the current study (32%) than reported for other free-living (or recently captive) Accipitridae, including *M. migrans* (36%), nestling osprey (*Pandion haliaetus*) (40%), and nestling golden eagles (39.5%).[12,14,15] However, a mean PCV value of 26.2% was reported for captive California condor (*Gymnogyps californianus*) below the age of 30 d, with this value increasing to 33.3% in birds between 1 and 6 mon of age, and again to 41.9% in birds between 6 mon and 5 yr of age.[7] The WBC values were generally higher in red kites in the current study than previously reported for red kites or other Accipitridae. For example, the median WBC count reported for *M. milvus* was nearly double that of black-shouldered and brahminy kites and nestling golden eagles, as well as being more than triple that reported for nestling osprey,[12,14,15] although the RI and mean were comparable to that reported from 34 captive California condors of varying ages, and only mildly higher than the mean values reported for nestling red kites in Germany.[19] Eosinophil and lymphocyte counts were also higher than reported for other nestling raptors, including red kites.[12,14,15,19] Although species and geographic differences are likely to exist, it is suspected that other factors played a role in this case.

This study population of nestling red kites had been in captivity for between 1 and 4 d at the time of sampling, unlike other referenced studies in which raptors were sampled from wild nests or had been held in captivity for longer. It is possible that the high WBC counts identified in these red kites are reflective of the effects of the stress of capture on the immune system, particularly because the red kites were assessed as being clinically well at the time of sampling.

It is likely that a proportion of free-living wild animals are suffering from underlying subclinical disease at any time, and it is important that RIs reflect this paradigm. Clinical findings were carefully considered alongside the results of statistical analysis to establish appropriate outliers to remove from the study group. This ensured the production of RIs applicable for use in future translocations of wild-born nestling kites under recently captive conditions. Five of six birds that were noted to be infected with *Leucocytozoon* sp. on examination of blood smear were not removed from the study population. *Leucocytozoon* spp. are avian hemoparasites that are commonly detected in nestling raptors, particularly those dwelling in forests, as they are transmitted by blackfly vectors that are present within these environments.[4,19] In most cases, *Leucocytozoon* spp. are of no clinical significance and do not precipitate disease.[19] Wiegmann et al (2021) detected this parasite in the blood of 51.9% (274 of 528) of clinically well free-living nestling goshawks (*Accipiter gentilis*), red kites, and common buzzards (*Buteo buteo*) tested in Germany. The authors also compared blood various blood parameters between infected and uninfected individuals and found that infected nestlings displayed significantly increased levels of heterophils, aspartate aminotransferase, and bile acids but decreased lymphocytes and monocytes compared with uninfected individuals. However, all differences reported were within the considered physiologic range.[19] In the present study, five of the *Leucocytozoon* infected red kites were not statistically noted to be outliers. For this reason, they were considered representative of the "normal" population and remained in the study group. One bird (37) had a high burden of *Leucocytozoon* sp. macro- and microgametes and was also identified as an outlier statistically on at least five parameters, justifying removal.

This study is not without limitations; mean cell volume and hemoglobin concentration were not examined and should be included as hematologic measures in future studies. Moreover, the red kites included in the study population were assessed as healthy based on a combination of clinical examination in field conditions and assessment of blood by a veterinary surgeon. More thorough diagnostic measures, such as radiography, were not performed, so individuals with subtle underlying disease may have been included.

The RIs presented in this study are representative of free-living red kite nestlings in England that have recently been captured for conservation translocation purposes. For this reason, they are likely to be particularly useful for future conservation interventions involving this species that have been undertaken in a similar manner for several decades and are likely to continue in the future. It is important that these values are considered alongside results of a thorough clinical examination, as it is possible that some clinically normal individuals will fall outside these ranges.

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## LITERATURE CITED

1. Balmer DE, Gillings S, Caffrey BJ, Swann RL, Downie IS, Fuller RJ. Bird atlas 2007–11: the breeding and wintering birds of Britain and Ireland. Thetford (UK): British Trust for Ornithology; 2013.

2. Brinc D, Khun M, Venner AA, Pasic MD, Colantonio D, Kyriakopolou L, Adeli K. Long-term stability of biochemical markers in pediatric serum specimens stored at −80°C: a CALIPER substudy. Clin Biochem. 2012;45(10–11):816–826.

3. Carter I. The red kite. Shrewsbury (UK): Arlequin Press; 2007.

4. Chakarov N, Veiga J, Ruiz-Arrondo I, Valera F. Atypical behavior of a black fly species connects cavity-nesting birds with generalist blood parasites in an arid area of Spain. Parasit Vectors. 2021;14(1):298.

5. Cray C, Rodriguez M, Zaias J, Altman NH. Effects of storage temperature and time on clinical biochemical parameters from rat serum. J Am Assoc Lab Anim Sci. 2009;48(2):202–204.

6. Dixon WJ. Processing data for outliers. Biometrics. 1953;9(1):74–89.

7. Dujowich M, Mazet J, Zuba J. Hematologic and biochemical reference ranges for captive California condors (*Gymnogyps californianus*). J Zoo Wildl Med. 2005;36(4):590–597.

8. Finnegan D. Reference intervals. R package version 1.2.0. Cran Repository [Internet]. 2015. https://cran.r-project.org/web/packages/referenceIntervals/referenceIntervals.pdf

9. Friedrichs KR, Harr KE, Freeman KP, Szladovits B, Walton RM, Barnhart KF, Blanco-Chavez J. ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics. Vet Clin Pathol. 2012;41(4):441–453.

10. Hernandez M, Margalida A. Hematology and blood chemistry reference values and age-related changes in wild bearded vultures (*Gypaetus barbatus*). J Wildl Dis. 2010;46(2):390–400.

11. Lopez-Lopez P, de la Puente J, Bermejo A, Urios V. Home range size and spatial ecology of adult red kites during the breeding season in Spain: preliminary results. In: II Int Symp Red Kite; 2015.

12. Meredith A, Surguine K, Handel I, Bronsvoort M, Beard P, Thornton SM, Wesche P, Hart M, Anderson D, Dennis R. Hematologic and biochemical reference intervals for wild osprey nestlings (*Pandion haliaetus*). J Zoo Wildl Med. 2012;43(3):459–465.

13. Montolio S, Valera R, Gonzalez S, Cray C, Lopez R, Ferron E, Francisco O, Sanches I, Casas-Diaz E. Plasma biochemistry RIs and age effect in European Strigiformes. Vet Clin Pathol. 2018;47(1):78–93.

14. Peniche G, Shaw D, Thompson D, Brain J, Reid R, Weston E, Benn S, Anderson D, Grant J, Pate L, Anderson NE, Meredith AL. Establishing haematological and biochemical reference intervals for free-ranging Scottish golden eagle nestlings (*Aquila chrysaetos*). Eur J Wildl Res. 2022;68(43):1–12.

15. Salakij C, Kasorndorkbua C, Pornpanom P, Salakij J, Jakthong P. Quantitative and qualitative characteristics of blood cells in black-shouldered, Brahminy, and black kites. Vet Clin Pathol. 2019;48(1):19–30.

16. Sergio F, Tanferna A, Chicano J, Blas J, Tavecchia G, Hiraldo F. Protected areas under pressure: decline, redistribution, local eradication and projected extinction of a threatened predator, the red kite, in Doñana National Park, Spain. Endanger Species Res. 2019;38:189–204.

17. Stevens M, Murn C, Hennessey R. Population change of red kites *Milvus milvus* in central southern England between 2011 and 2016 derived from line transect surveys and multiple covariate distance sampling. Acta Ornithol. 2020;54(2):243–254.

18. Taylor EC, Sethi B. Stability of 27 biochemistry analytes in storage at a range of temperatures after centrifugation. Br J Biomedic Sci. 2011;68(3):147–157.

19. Wiegmann A, Springer A, Rinaud T, Ottensmann M, Legler M, Krüger O, Fehr M, Chakarov N, Strube C. The prevalence of *Leucocytozoon* spp. in nestlings of three wild raptor species including implications on haematological and blood chemistry values. Int J Parasitol Parasites Wildl. 2021;16:236–243.

20. Veterinary Surgeons Act 1966. Chapter 36. Available at: www.legislation.gov.uk/ukpga/1966/36 (Accessed: 9 September 2023).

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