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Original Study

Erythrocyte Sedimentation Rate Is a Promising Screening Test for Infections in Rehabilitated Birds

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Abstract: Erythrocyte sedimentation rate (ESR) is a simple, inexpensive test that is an indirect measure of the acute-phase protein response to inflammation. Acute-phase assays can increase detection of illnesses when used in conjunction with other hematological tests, and may be more sensitive to some diseases than white blood cell counts. This study assessed the usefulness of testing ESR with a microhematocrit tube for detecting inflammatory and infectious conditions in rehabilitated birds. Values were measured in 119 clinically healthy birds from 5 orders, and differences between ages, orders, and healthy and unhealthy birds were compared. Cutoff values to differentiate clinically normal birds from those with trauma or infections were done by receiver operating characteristic curves, nonparametric methods, and a linear regression–based method to account for differences in packed cell volume (PCV). The ESRs of unhealthy birds (n = 188) were used to assess performance of the different cutoff methods. The receiver operating characteristic curve cutoffs for infected birds in Accipitriformes, in Strigiformes, and for combined orders had good sensitivity (78–97%), specificity (94–100%), and area under the curve values (0.913–0.990), but performance was likely overestimated due to sample size limitations. There was a significant negative correlation between PCV and ESR, but regression-based cutoffs had the worst overall performance, and all methods had low sensitivity for unhealthy passerines. Erythrocyte sedimentation rate appears to be a useful test in some orders, can be easily implemented, and can be performed with the same sample used to determine PCV and total protein, increasing diagnostic information without the need for additional blood or testing costs. However, species-, age-, and sex-specific differences must be further investigated to fully assess the usefulness of this test.

Key words: inflammation, infection, erythrocyte sedimentation rate, acute-phase proteins, screening test, hematology, avian

INTRODUCTION

The acute-phase response is a part of the innate immune system that responds to inflammatory conditions such as infection, trauma, stress, neoplasia, and autoimmune disease.[1,2] Acute-phase assays, such as the erythrocyte sedimentation rate (ESR), are used to identify disease and monitor progress of inflammation, and can aid in prognosis.[3] Measurement of acute-phase proteins has been found to be more sensitive than white blood cell (WBC) counts to changes in inflammation in numerous conditions and species.[1] The ESR is a simple test that is an indirect measure of the acute-phase response, determined by the distance that red blood cells settle out of plasma in 1 hour, with increased distances associated with inflammation. It is an indirect but inexpensive test that may be used to identify negative health conditions. Sedimentation rate has been demonstrated to be a useful indicator of reduced health or disease in multiple avian species, such as nephritis in poultry, tick parasitism in great tits, leg deformities in emus, and reduced health in blue tits and hooded crows.[3–7]

One issue with traditional ESR tests is that they require 0.6–2 mL of whole blood, which can be impractical for smaller avian species. Several studies have measured ESR with microhematocrit tubes in chelonian species, demonstrating its applicability for use in small wildlife species.[8,9] This method uses less than 0.1 mL of blood, is useful with its own reference interval, and can be easily done as a part of routine diagnostic testing on rehabilitated birds. Additionally, cost is often a limiting factor for diagnostics in wildlife rehabilitation clinics, and this method of testing

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ESR is very inexpensive.[10] Despite this, ESR has not been studied in most avian species and is rarely referenced as a part of health assessment.

In conjunction with a physical examination, diagnostic testing that use blood, such as packed cell volume (PCV)/total protein (TP) and a WBC count and differential, are useful and commonly performed as a part of physically assessing rehabilitation patients.[11] White blood cell counts are the main indicator of infection or inflammation but have several limitations. If the WBC count is done by the estimation method from a slide, it can be difficult to perform in severely anemic birds, and manual complete blood cell count (CBC) results may vary by technician.[12] Additionally, reference intervals vary widely between species, species-specific reference intervals may not be available, and reference intervals can vary depending on source.[11,12] Similarly, the stress leukogram may also vary significantly between species, affecting the WBC count and differential.[12] Finally, WBC counts may not always be reliable indicators of illness, and ESR, acute-phase proteins, or plasma electrophoresis may be abnormal when the CBC is not.[13,14] Use of the ESR may help to increase diagnostic sensitivity to inflammatory conditions in birds without obvious abnormalities on the physical examination or CBC.

The purpose of the study reported here was to determine whether the microhematocrit ESR method is useful as an inexpensive screening test for inflammatory or infectious conditions in rehabilitated birds. Its primary focus was whether the test could be useful with minimal equipment investment, with a blood sample that is regularly collected as a part of health assessment. Because it is difficult to establish reference intervals for many wildlife species due to limited numbers of individuals available for sampling, a major goal of this study was to investigate whether extrapolating across multiple species with PCV is possible.[12] This was done by establishing a generalized set of ESR reference intervals from multiple orders, as well as ESR reference intervals from different orders for comparison. Lastly, I evaluated the sensitivity and specificity of the test for a variety of traumatic (eg, fractures, central nervous system) and infectious (eg, bacterial, parasitic) conditions by different statistical methods for generating cutoff values between healthy and diseased birds. I hypothesized that ESR could be extrapolated between different species due to the effect of PCV on sedimentation rate, and that ESR's would be increased in birds with injuries or illnesses compared to values from healthy birds.

MATERIALS AND METHODS

Sedimentation rate was measured by the microhematocrit method described by Adamovicz et al[8] for use in free-living box turtles (*Terrapene* spp). Blood was drawn from the basilic, medial metatarsal, or jugular vein with a 25- or 27-gauge needle. A microhematocrit tube was filled approximately 80% full, plugged with clay, and set vertically in a stand with a level. A timer was then set for 60 minutes, and the distance from the top of the plasma to the top of the red blood cells was measured with digital calipers (6 inch [15-cm] 3 Mode Digital Fractional Calipers, Husky Corporation, Pacific, MO, USA) within 2 minutes of the end of the timing period. Temperature during ESR measurements was approximately 20–21.1°C (68–70°F). After measurement of the ESR, samples were centrifuged at 3,400 RPM for 5 minutes with a Clay Adams 0151 Analytical Centrifuge (Parsipanny, NJ, USA) and PCV was measured using a Critocaps microhematocrit capillary tube reader (Oxford Labware, St. Louis, MO, USA). All sampling was performed at the Vermont Institute of Natural Science (VINS; Quechee, VT, USA) between December 30, 2023 and December 15, 2024. Sampling of different species, ages, and injury or infection types was limited by the individuals presented for rehabilitation.

Reference ESR intervals were measured using samples from rehabilitated birds in the prerelease period, birds that were presented for rehabilitation without a diagnosed injury or illness (ie, birds that arrived as healthy fledglings), or from birds with stable, nonreleasable injuries (eg, old eye injuries, healed fractures) that were resident birds. Samples were also collected from nonreleasable rehabilitation patients that were clinically healthy aside from nonreleasable injuries after they had been anesthetized for euthanasia. Blood samples from healthy birds were collected once per individual. A minimum target of 120 individuals was set for establishing a set of reference ESR intervals, according to the minimum sample size recommended by the American Society of Veterinary Clinical Pathologists to account for multiple sexes and ages among a species, while acknowledging that this is not a true reference interval given the numerous species that were sampled to establish it.[12] Sex is not routinely recorded at VINS unless there is obvious plumage or size dimorphism, so sex was not recorded for most individuals and was not included in statistical analysis.

Samples from unhealthy individuals were collected as a routine part of health assessments when presented or if warranted while in care. Only 1 sample was included for each unhealthy individual, and only

Table 1. Diagnostic criteria for traumatic injuries in rehabilitated birds. Each category of traumatic injury (eg, central nervous system, musculoskeletal, internal) is listed with the various methods used for diagnosis, as well as the number of cases diagnosed by each method. Some birds may have had multiple injuries diagnosed with different methods (eg, fundic examination and radiographic abnormalities).

Category Central nervous system trauma	Methods used for diagnosis	Cases
Head/ocular trauma	Mentation or neurological abnormalities with evidence or history of trauma	96
	Abnormalities on visual or fundic exam; fluorescein stain; cranial nerve abnormalities	
Spinal trauma	Paralysis or paresis; altered withdrawal or deep pain reflex	4
	Radiographic evidence of spinal cord injury	6
Musculoskeletal trauma	Soft tissue injuries or fractures on physical examination	69
	Radiographic evidence of fractures	27
Internal trauma	Radiographic evidence (internal bleeding, organ displacement, keel fractures)	5
	Traumatic injuries and at least 2 of the following:	17
	Bruising or subcutaneous emphysema on thorax or abdomen	
	Dyspnea or wet breath sounds	
	Blood from the glottis or vent	
	Moderate to marked anemia without external evidence of bleeding	

the ESR from the initial blood collection was used. Physical examinations were performed by a staff with 7–10 years of avian rehabilitation experience. Depending on the case, further diagnostic testing may have included PCV/TP, CBC, plasma biochemistry panel, radiographic imaging, fecal flotation for parasite evaluation, cytology, and/or postmortem examinations. Fundic exams were performed on all raptors, crows, and ravens, but not consistently for small passerines. For ESR assessment, traumatic injuries were classified as CNS (head, spine, ocular trauma), musculoskeletal (fractures, wounds), internal, or mixed trauma. Infections were classified as bacterial, fungal, parasitic, viral, or mixed. Some cases were classified as mixed trauma and infection. Criteria for diagnosis of trauma can be seen in Table 1, and criteria for diagnosis of infections can be seen in Table 2. Birds with toxicities or no clear diagnosis of trauma or infection were excluded from the study. All unhealthy birds that could be sampled during the sampling period as healthy birds and were not excluded based on the above criteria were included. No target sample size for unhealthy birds was established.

Sedimentation rate for all orders combined (Accipitriformes, Passeriformes, and Strigiformes) was evaluated for differences and usefulness in detecting trauma or infections. The Tukey outlier test was used to eliminate outliers in groups of healthy birds. Columbiformes ($n = 10$) and Falconiformes ($n = 5$) had too few individuals for useful interpretation, so no further analysis was performed on these orders. Age classes were designated as nestling/fledgling (N/F), hatch year (HY), or after hatch year (AHY). Median and interquartile range (IQR) were generated for ESR and PCV for each healthy group, as well as their respective trauma and infection comparison groups. The Kruskal-Wallis test with Dunn post hoc comparisons was used to evaluate differences in ESR and PCV between taxonomic orders

Table 2. Criteria used for diagnosing different infections (eg, bacterial, fungal, viral, parasitic, and mixed/unspecified) in birds at the Vermont Institute of Natural Science (Quechee, VT, USA). Methods are listed for each category, as well as the number of cases diagnosed by each method. Some birds were diagnosed with multiple methods (ie, gastrointestinal parasitism with diarrhea and fungal infection diagnosed at necropsy).

Category and method	Cases
Bacterial	
Cytology	2
Necropsy with culture	2
Necropsy	2
Chronic/necrotic wounds/open fractures	14
Fungal	
Moldlike or “bullseye” lesions at necropsy	7
Pox virus	
Characteristic lesions on unfeathered areas	4
Parasitic	
Myiasis	6
Positive fecal float with diarrhea and weight loss	7
Cytology	5
Blood smears	4
Mixed/unspecified	
CBC (leukocytosis with toxic and/or left shift)	7
CBC with radiographs (respiratory infections)	2
Necropsy	14
Histopathology	1

Abbreviation: CBC, complete blood count.

and age classes. Multiple regression was not used to compare effects of PCV and taxonomy or age due to multicollinearity detected by Pearson *r* test. Cutoff values to differentiate between healthy and unhealthy individuals were generated in 3 ways to compare sensitivity and specificity of each method. The first method used the 97.5th percentile as the upper cutoff from each group of healthy birds ("nonparametric cutoff"). The second method used nonparametric receiver operating characteristic (ROC) curves to generate optimal cutoff values by the Youden J statistic, as well as area under the curve (AUC) values to evaluate overall usefulness of the test. All available data were used for ROC analysis. Sedimentation rate has been shown to be affected significantly by PCV, so the third method used the upper limit of 95% prediction intervals from the linear regression line of healthy values to generate cutoffs that account for differences in PCV.[15] The upper limit was calculated for 5% intervals of PCV (eg, 30%, 35%) to create a series of cutoff values based on PCV. The combined-orders group was used to generate this model, to help assess whether PCV based extrapolation may be clinically useful. Regression analysis was used to assess the effects of how PCV and different types of traumatic injury (eg, central nervous system, musculoskeletal, internal) affected changes in ESR. Data for multiple regression were log transformed to achieve normal residuals, then back transformed for reporting of coefficients. Multiple regression was not performed for infections because the types of infections present were often less clearly defined. The Shapiro-Wilk test and residual plots were assessed for regression analysis to test for normality and heteroscedasticity. A table of the sensitivity and specificity using nonparametric cutoffs, ROC cutoffs, and regression cutoffs for trauma and infections for each group was made for comparison.

Receiver operating curves, AUC, and cutoff values were calculated by easyROC (version 1.3.1, Erciyes University, Kayseri, Turkey). Kruskal-Wallis tests, Dunn post hoc comparisons, Pearson *r* values, regression analysis, and Shapiro-Wilk tests were performed in Jeffrey's Amazing Statistics Program (version 0.19.1, University of Amsterdam, Amsterdam, The Netherlands). All other statistical analyses were performed by Microsoft Excel 2019 (Microsoft, Redmond, WA, USA). The experimental protocols were approved by VINS institutional animal care and use committee (protocol number: 24-06). All rehabilitation work was performed with appropriate state (WR-2025-15) and federal (MB695060) rehabilitation permits.

RESULTS

Erythrocyte sedimentation rate reference intervals were established from 119 clinically healthy individuals comprising 21 species and 5 orders. Erythrocyte sedimentation rates were also obtained from birds considered unhealthy with traumatic injuries (128 birds, 23 species) and infections (60 birds, 21 species); these birds were from the same 5 orders as the clinically healthy individuals (Table 3, Supplemental Table 1).

Erythrocyte sedimentation rate values of clinically healthy birds tested for outliers had 2 values removed from Accipitriformes and 1 value each removed from Columbiformes, Falconiformes, and Strigiformes. Range, median, and IQR of ESR and PCV for healthy and unhealthy birds from each taxonomic group and all orders combined were calculated (Table 4). Accipitriformes had a significantly lower ESR than Passeriformes ($P = 0.004$) and Strigiformes ($P < 0.001$) and a significantly lower PCV than Strigiformes ($P = 0.011$). Passeriformes had a significantly higher PCV than Strigiformes ($P = 0.048$).

When age data were compared, N/F birds had a significantly higher ESR than HY ($P = 0.002$) or AHY birds ($P < 0.001$). Nestlings/fledglings also had a significantly lower PCV than AHY birds ($P < 0.001$), but not from HY birds ($P = 0.09$), and there was no significant difference in PCV between HY and AHY birds ($P = 0.16$). Median, IQR, and range of ESR and PCV were calculated for each age group (Table 5).

Linear regression showed a significant negative correlation between PCV and ESR (adjusted $R^2 = 0.578$, $\beta = -0.763$, $P < 0.001$). The multiple regression model for PCV and traumatic injury effects on ESR was statistically significant (adjusted $R^2 = 0.552$, $P < 0.001$). It found that PCV ($\beta = -0.651$, $P < 0.001$) and internal injuries ($\beta = 0.192$, $P = 0.005$) had significant effects on ESR. Central nervous system trauma ($P = 0.80$) and musculoskeletal injuries ($P = 0.60$) had no significant effect on ESR in the regression model. Because there was no significant effect of the latter 2 variables in the model, birds with infections that also had CNS or musculoskeletal trauma were placed in the infection group for ROC analysis.

Cutoff values generated by ROC curves had the best overall performance, but performance of ROC and nonparametric cutoffs was similar in Accipitriformes because both methods generated a very similar cutoff value (Fig 1). Sensitivity and specificity for infections and the AUC (0.8–1) from ROC cutoffs were generally good, with the exception of Passeriformes (Table 6). Sensitivity was generally low for

Table 3. A summary of individuals sampled for erythrocyte sedimentation rate by order and health status. The number of nestling/fledgling, hatch-year, after-hatch-year, and unknown age birds, the number and percentage of total individuals for different health categories in each order, and the total number of individuals for each category are shown.

Group	Species, n	N/F, n (%)	HY, n (%)	AHY, n (%)	Unk, n (%)	Total
Accipitriformes						
Healthy	6	0 (0)	13 (39.4)	20 (60.6)	0 (0)	33
Trauma	8	1 (2.7)	20 (54.0)	16 (43.3)	0 (0)	37
Infection	8	6 (18.2)	18 (54.5)	9 (27.3)	0 (0)	33
Columbiformes						
Healthy	2	2 (18.2)	3 (27.3)	6 (54.5)	0 (0)	11
Trauma	2	0 (0)	0 (0)	4 (100)	0 (0)	4
Infection	2	1 (50)	1 (50)	0 (0)	0 (0)	2
Falconiformes						
Healthy	3	2 (33.3)	0 (0)	3 (50)	1 (16.7)	6
Trauma	2	0 (0)	1 (25)	2 (50)	1 (25)	3
Infection	1	0 (0)	1 (25)	0 (0)	0 (0)	2
Passeriformes						
Healthy	7	22 (59.5)	2 (8.1)	9 (24.3)	2 (8.1)	37
Trauma	7	10 (38.5)	4 (15.4)	10 (38.5)	2 (7.6)	26
Infection	7	6 (46.2)	2 (15.4)	4 (30.8)	1 (7.6)	13
Strigiformes						
Healthy	3	0 (0)	2 (6.7)	7 (23.3)	23 (70)	32
Trauma	3	1 (1.7)	4 (6.9)	21 (36.2)	32 (55.2)	58
Infection	3	1 (10)	1 (10)	4 (40)	4 (40)	10

Abbreviations: n, number; N/F, nestling/fledgling; HY, hatch-year; AHY, after-hatch-year; Unk, unknown.

trauma regardless of method used, except for Accipitriformes. Nonparametric cutoffs had high specificity, but often low sensitivity. The regression-based cutoff method generally performed worse than the other 2 methods, only showing superior performance for infected Passeriformes; however, sensitivity in this group was still poor (Table 7). Values above the reported cutoff at a given PCV may be elevated; for example, an ESR over 2.50 mm may be elevated at a PCV of 45% (Figs 2 and 3).

DISCUSSION

The results of this study show that ESR testing with microhematocrit tubes has promise as a screening test for infections in rehabilitated birds, but further work is needed to establish species-, age-, and sex-specific reference intervals and validation of cutoff values. There were good sensitivity, specificity, and AUCs for infections in Accipitriformes, Strigiformes, and combined orders.[16] Cutoff values by ROC methods had the best overall performance, whereas nonparametric cutoffs had lower sensitivity but high specificity, and the regression-based method generally performed worse than the other methods. When considering that there is relatively little cost for each test aside from the initial investment of digital calipers ($20–40) and that the same sample can be used for measuring PCV/TP, the performance for infection screening is quite good when considering the cost.

Although optimal values from ROC analysis had the best overall performance, regression-based cutoffs may be of use at low or high PCVs. For example, a bird with anemia due to emaciation would have an increased ESR compared with a healthy bird, but use of the regression line–based cutoffs might show increases beyond what is expected due to the effect of a decreased PCV. For example, an emaciated barred owl (*Strix varia*) with a PCV of 24% and an oropharyngeal bacterial infection diagnosed cytologically had an ESR of 5.67 mm, whereas the healthy cutoff based solely on changes to PCV provided by the regression line was approximately 4.16 mm. It may also provide increased sensitivity at PCVs over 45–50%, where an ESR may be below cutoffs from other methods; however, it may be increased compared with what would be expected at that PCV in a healthy bird. Regression-based cutoffs based on PCV may also allow for approximation of healthy ESR when extrapolating to species without established reference intervals, but should be interpreted carefully because differences between species, ages, and sexes are likely common, and they showed the worst performance of all the methods used in this study. Their

Table 4. Medians, interquartile ranges (IQRs), and ranges for erythrocyte sedimentation rates and packed cell volumes were calculated for each taxonomic group and all 5 orders combined. The number of individuals in each category, medians, IQRs, and ranges of erythrocyte sedimentation rates and packed cell volumes for healthy birds, birds with trauma, and birds with infections are shown. Median and IQRs are not shown for unhealthy Columbiformes and Falconiformes due to small sample sizes.

		ESR			PCV		
Group	n	Median	IQR	Range	Median	IQR	Range
Accipitriformes							
Healthy	33	0.97	0.72–1.2	0.41–2.15	47	43–49	39–60
Trauma	37	2.65	1.86–4.19	0.55–14.5	38	32–43	16–54
Infection	33	4.6	3.04–9.72	1.18–47.67	30	27–35	13–50
Columbiformes							
Healthy	11	0.72	0.57–0.81	0.43–1.84	56	52–58	37–59
Trauma	4			0.36–3.32			29–57
Infection	2			0.59–6.09			38–47
Falconiformes							
Healthy	5	1.24	1.07–1.36	1.07–1.62	46	46–49	38–50
Trauma	4			1.08–2.26			45–47
Infection	1			4.53			42
Passeriformes							
Healthy	37	1.32	0.82–1.83	0.5–2.72	46	43–49	35–53
Trauma	26	1.84	1.14–3.26	0.56–7.20	41	33–46	9.0–50
Infection	13	2.41	1.51–3.33	0.37–6.72	41	34–44	30–56
Strigiformes							
Healthy	32	1.36	1.12–1.77	0.84–2.71	44	42–46	39–52
Trauma	58	2.84	1.67–4.16	0.93–13.64	38	29–42	16–49
Infection	10	4.23	3.62–5.97	1.92–6.91	27	26–30	18–43
Combined orders							
Healthy	119	1.18	0.82–1.69	0.41–3.01	46	42–49	34–60
Trauma	129	2.32	1.59–3.8	0.36–14.5	38	31–43	9.0–57
Infection	61	4.06	2.41–6.09	0.37–47.67	32	27–38	13–56

Abbreviations: n, number; ESR, erythrocyte sedimentation rates; PCV, packed cell volumes; IQR, interquartile range.

accuracy would likely be increased if calculated for specific orders or species, rather than the multispecies cutoffs generated in this study.

Sedimentation rate does not appear to be a useful test for trauma, potentially due to the time delay before the rate increases when birds with very recent trauma are assessed.[2] Elevated values, particularly when anemia is present, may be suggestive of internal trauma. Trauma, however, is often easier to diagnose when a patient is physically examined, especially if other diagnostic testing (eg, radiographic imaging, ophthalmic examination) is included, than underlying infections without obvious external clinical disease signs.

As a measure of the acute-phase response, ESR implies a decreased albumin to globulin ratio because albumin is the major negative acute-phase protein and globulins are positive acute-phase proteins.[1] Because it is highly nonspecific, increases should be an indication to perform other diagnostic tests

Table 5. A comparison of erythrocyte sedimentation rates and packed cell volumes between different age groups of healthy birds. Sample sizes, medians, interquartile ranges, and ranges for erythrocyte sedimentation rates and packed cell volumes are shown for nestlings/fledglings, hatch year, and after hatch year birds. Birds of unknown age (n = 27) are not shown.

		ESR			PCV		
Age	n	Median	IQR	Range	Median	IQR	Range
N/F	27	1.75	1.4–1.9	0.82–2.72	44	40–47	35–49
HY	20	1.08	0.79–1.57	0.55–3.01	46	43–48	34–56
AHY	45	0.87	0.71–1.17	0.41–2.9	49	44–51	39–60

Abbreviations: n, number; IQR, interquartile range; ESF, erythrocyte sedimentation rate; PCV, packed cell volume; N/F, nestlings/fledglings; HY, hatch year; AHY, after hatch year (AHY).

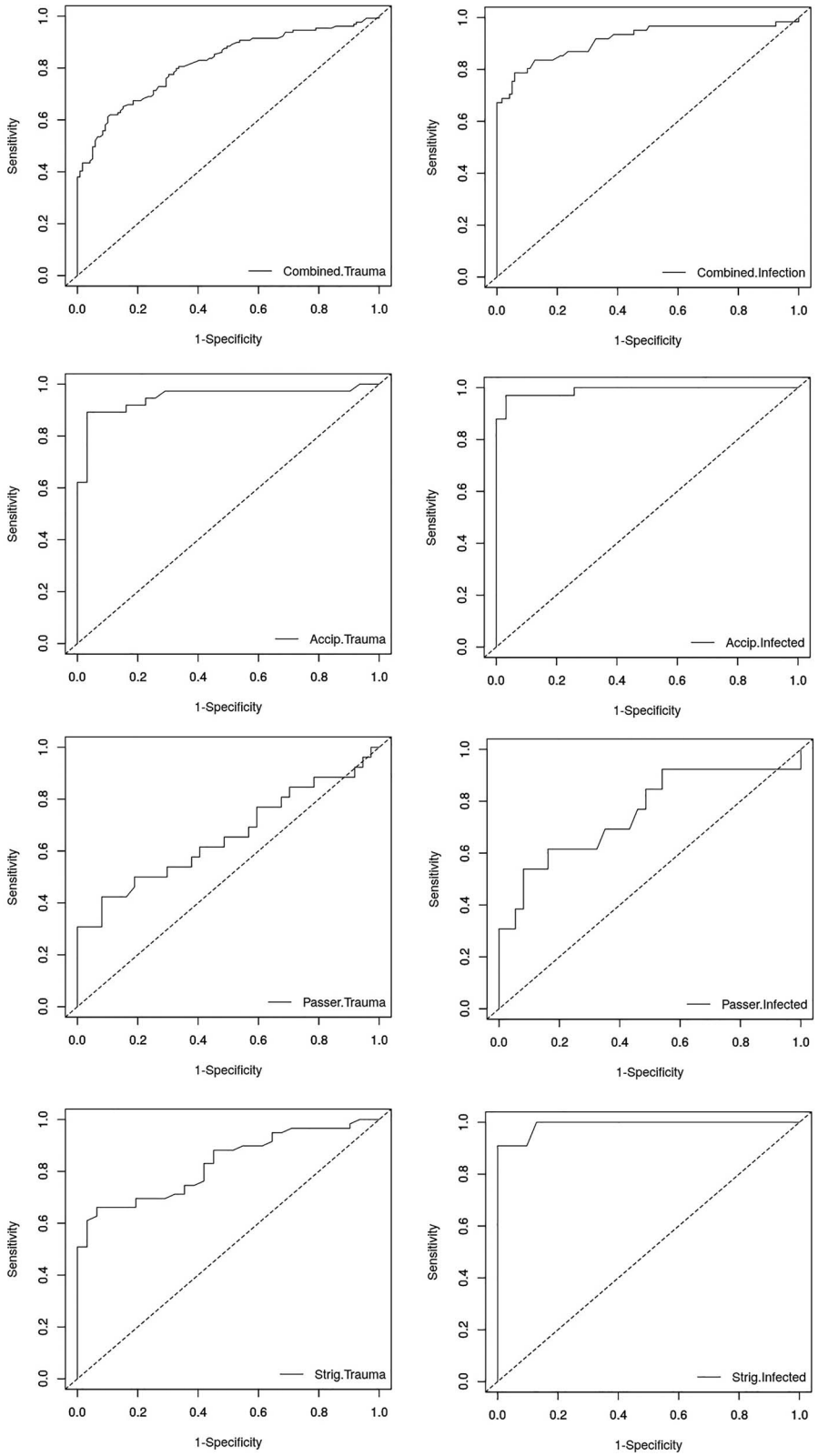

Figure 1. Comparisons of receiver operating characteristic curves of erythrocyte sedimentation rates from unhealthy birds from different taxonomic groups. Curves from trauma and infection cases are shown for combined orders. Abbreviations: Accip., Accipitriformes; Passer., Passeriformes; Strig., Strigiformes.

Table 6. Sensitivities, specificities, cutoff values, and receiver operating characteristic (ROC) statistics for erythrocyte sedimentation rate measurements between healthy and unhealthy birds in different taxonomic groups. Cutoff values with their respective sensitivities and specificities are shown for the ROC and nonparametric methods. Only sensitivity and specificity are shown for the regression line method because the cutoff values are dependent on packed cell volume. The areas under the curve for different categories are shown for ROC cutoffs, as well as the number of unhealthy individuals in each category.

Category	Cutoff method	Cutoff, mm	Sensitivity, %	Specificity, %	AUC	Unhealthy, n
Combined trauma	ROC	1.96	61.2 (52.3–69.7)	89.9 (83.0–94.7)	0.819 (0.77–0.87)	129
	Nonparametric	2.72	43.4	98.3	—	
	Regression Line	—	36.9	97.5	—	
Combined infection	ROC	2.27	78.7 (66.3–88.1)	94.1 (88.3–97.6)	0.913 (0.86–0.97)	61
	Nonparametric	2.72	68.9	98.3	—	
	Regression line	—	62.3	97.5	—	
Accipitriformes trauma	ROC	1.50	89.2 (74.6–97.0)	96.8 (83.3–99.9)	0.949 (0.89–1.01)	37
	Nonparametric	1.52	86.5	93.5	—	
	Regression line	—	51.4	96.8	—	
Accipitriformes infection	ROC	1.47	97.0 (84.2–99.9)	96.8 (83.2–99.9)	0.989 (0.97–1.01)	33
	Nonparametric	1.52	93.9	93.5	—	
	Regression line		69.7	96.8	—	
Passeriformes trauma	ROC	2.46	42.3 (23.4–63.1)	91.9 (78.1–93.3)	0.656 (0.51–0.80)	26
	Nonparametric	2.72	30.8	94.6	—	
	Regression line	—	7.7	94.6	—	
Passeriformes infection	ROC	2.41	53.8 (25.1–80.8)	91.9 (78.1–93.3)	0.755 (0.58–0.93)	13
	Nonparametric	2.72	33.3	94.6	—	
	Regression line	—	38.5	94.6	—	
Strigiformes trauma	ROC	1.96	62.1 (52.6–77.9)	93.5 (78.6–99.2)	0.828 (0.75–0.91)	59
	Nonparametric	—	57.6	96.8	—	
	Regression line	—	39.0	97.2	—	
Strigiformes infection	ROC	3.28	90.9 (58.7–99.8)	100 (88.8–100)	0.990 (0.97–1.01)	11
	Nonparametric	—	91.7	96.8	—	
	Regression line	—	72.7	97.2	—	

Abbreviations: mm, millimeters; n, number; AUC, area under the curves; ROC, receiver operating characteristics.

Table 7. Erythrocyte sedimentation rate (ESR) cutoffs for different packed cell volumes (PCVs). These values were generated by linear regression to account for how changes in PCV affect ESR. Values above an ESR at its respective PCV (eg, over 2.42 mm at 40% PCV) are considered elevated based on this method of generating cutoff values.

PCV, %	Cutoff, mm
5	5.88
10	5.45
15	5.01
20	4.58
25	4.16
30	3.74
35	3.32
40	2.90
45	2.50
50	2.09
55	1.70
60	1.30

Abbreviations: mm, millimeters; PCV, packed cell volume.

(eg, CBC, imaging, specific pathogen testing) to come to a more definitive diagnosis. As a part of a health screening test, ESR can be performed along with a PCV/TP and a blood smear, providing a substantial amount of information from a small (~0.1 mL) blood sample that can be collected from most birds. Combining acute-phase protein tests such as fibrinogen or C-reactive protein with a CBC or ESR has been shown to increase the sensitivity for conditions such as bacterial infections.[17,18] Therefore, the addition of ESR to a health screening test may increase the chance of detecting illness. Erythrocyte sedimentation rate is a quick and simple procedure, compared with tests such as the heat precipitation method for measuring fibrinogen, and can be performed by staff with minimal training.[19] Although this was not assessed in this study, it may also be of use in prognosis and monitoring progress of infections.

A critical focus area for further use of ESR in wild birds is the need to establish species-, age-, and

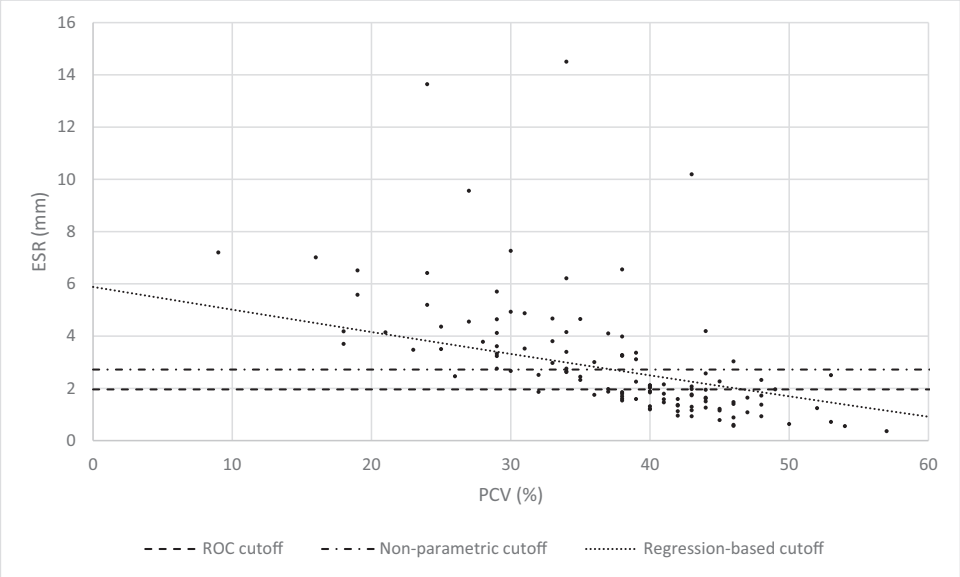

Figure 2. Distribution of erythrocyte sedimentation rates in birds with traumatic injuries from all orders included in the study. The receiver operating characteristic curves, nonparametric, and regression-based cutoffs are superimposed to show where values fall relative to each cutoff method. Abbreviations: ESR, erythrocyte sedimentation rates; ROC, receiver operating curves; PCV, packed cell volumes.

sex-specific reference intervals. Although the correlation to PCV may allow some estimate of expected ESR in a healthy bird, differences between taxonomic groups were detected in this study. For example, Passeriformes had significantly higher ESR than Accipitriformes, despite no significant difference in PCV. This may be due to Passeriformes having ESR values within reference intervals when healthy at a given PCV. However, it is hard to interpret how significant these differences are due to age-related sampling bias, with 59% of healthy Passeriformes being in the N/F age class and none of the Accipitriformes in this age class. The higher ESRs in passerines may be a consequence of many young birds included in

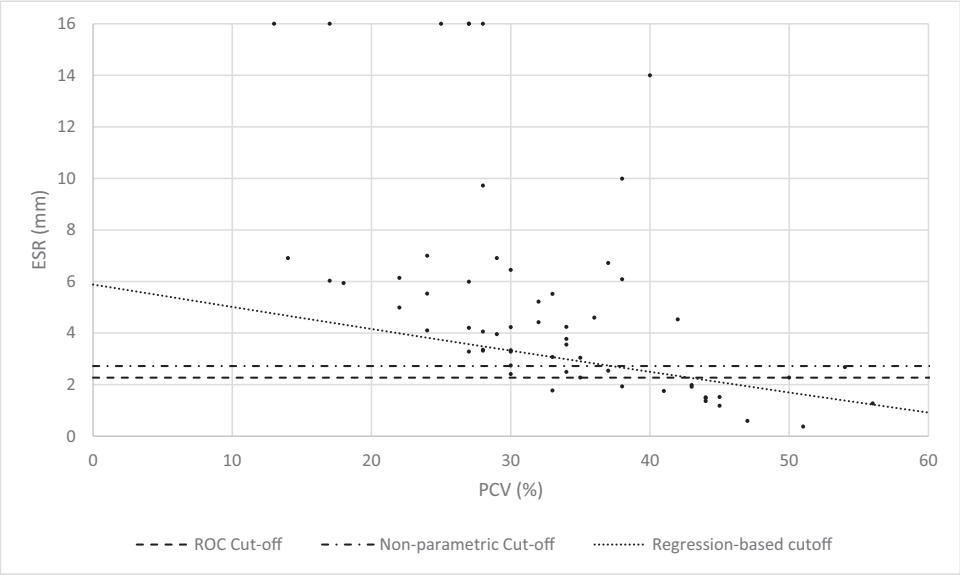

Figure 3. Distribution of erythrocyte sedimentation rates in birds with infections from all orders included in the study. The receiver operating characteristic curves, nonparametric, and regression-based cutoffs are superimposed to show where values fall relative to each cutoff method. Five data points at the top of the figure represent erythrocyte sedimentation rates with values over 16 mm. Abbreviations: ESR, erythrocyte sedimentation rates; ROC, receiver operating curves; PCV, packed cell volumes.

this study, as opposed to a taxonomic difference. The higher values in young birds could also be due to innate differences, or might be an immune response to new parasitic infection that adults with chronic, established infections might not exhibit. Despite these differences, the reference interval of ESR among raptors appears to remain small, whereas WBC counts in raptors can vary by a 10-fold degree in healthy individuals of different species.[20,21]

The lack of species-specific values and limited sample sizes for unhealthy birds in some categories are major limitations of this study. The taxonomic differences noted previously indicate a need for future research regarding interspecies differences. Further work and additional sampling to compare changes in unhealthy birds is needed to better assess the accuracy of ESR. For example, the ROC cutoff of 1.47 mm in Accipitriformes with infections in this study has a sensitivity and specificity of approximately 97%, which is almost certainly an overestimate of the test's performance. Anecdotal observations by the author suggest it may not be regularly elevated in viral infections, but this is based on a low number of mostly unconfirmed infections, and investigation of its performance for different infectious disease conditions would be useful.

Because this method of ESR is designed to be easily implemented with minimal investment, reduced precision is a potential tradeoff, although it appears more precise than commercially available Winpette tubes in another study.[8] A number of factors that can affect the ESR rate have been reported.[17] Vibrations and inclination of the blood-filled tubes can increase the rate, with even small changes in the angle leading to potentially significant changes in ESR. Despite this, using a level to set the tubes vertically appears to maintain consistent results. In birds with lower hematocrits, the reduced numbers of red blood cells may be more susceptible to artifactual changes in rate, which might lead to greater variance at lower PCVs. Warmer temperatures and sunlight can also increase the ESR, which is unlikely to be an issue in a clinic, but could affect values if used in the field. Finally, proper venipuncture technique and filling the tube in an appropriate manner to avoid clots and bubbles are important to minimize artifactual changes. Additional variables to assess include confirming that PCV/TP readings agree with samples that are immediately centrifuged versus samples centrifuged and measured after ESR is performed, as well as the effects of different anticoagulants and storage times, because ESR in this study was performed immediately after the blood was collected.

Although inexpensive, this test has some notable limitations. It is nonspecific, with a myriad of potential traumatic and infectious conditions that can lead to increases. It can also be normal in the presence of severe injury or illness, so users must remain aware of the potential of inaccurate results. For example, a juvenile broad-winged hawk (*Buteo platypterus*) with suspected sepsis or severe hepatitis (leukopenia with toxic left shift and hypoglycemia) had no elevation in ESR, potentially related to hypofibrinogenemia or liver disease.[22,23] Although the test shows some benefits with the generalized values, evaluating differences between species, age, and sex could increase its sensitivity and accuracy and should be a priority for future research.[6,8,17] Because ESR has also been used in chelonians, it could likely be utilized in a wide range of small reptilian and mammalian species. Evaluating changes in ESR due to specific disease conditions (eg, aspergillosis, salmonellosis, West Nile virus) could also increase its usefulness as a diagnostic screening test.

This method of ESR measurement has potential to be used in companion exotic animal medicine, zoo medicine, and wildlife field studies. It could easily be incorporated as an inexpensive part of a health screening test for domestic or wild birds in long-term captivity. Hematology is the most commonly used tool in wildlife health assessment, and ESR has been used to a limited degree in avian fieldwork but could be incorporated on a more regular basis.[24] Again, reference intervals are needed to better assess effects of species, age, and sex to better evaluate ESR changes from these variables. Reference intervals from species within the same family or genus would likely allow for better extrapolation of values than the regression-based method in this study.[12]

Despite the limitations discussed previously, ESR by the microhematocrit method is an inexpensive, easily implemented test that shows promising performance in detecting infections in some avian orders. Increases in the ESR may also be suggestive of internal problems in birds with traumatic injuries, possibly helping to guide treatment or additional diagnostics. Further work establishing species-, age-, and sex-specific reference values is needed to fully validate this test. Studies are required to assess how various illnesses affect ESR in birds, which could increase its usefulness, especially in small or sick birds. When trying to maximize the amount of information from very low-volume blood samples collected from small or sick birds, ESR can provide additional clinical information without critically endangering the patient. This inexpensive, easy-to-perform test can be readily implemented for a wide range of species in a variety of clinical settings, making it a useful addition when avian bloodwork is being performed.

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