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EXPLORING THE USE OF THE ERYTHROCYTE SEDIMENTATION RATE AS AN INFLAMMATORY MARKER FOR FREE-RANGING WILDLIFE: A CASE STUDY IN AFRICAN BUFFALO (*SYNCERUS CAFFER*)

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ABSTRACT: Measuring inflammatory markers is critical to evaluating both recent infection status and overall human and animal health; however, there are relatively few techniques that do not require specialized equipment or personnel for detecting inflammation among wildlife. Such techniques are useful in that they help determine individual and population-level inflammatory status without the infrastructure and reagents that many more-specific assays require. One such technique, known as the erythrocyte sedimentation rate (ESR), is a measure of how quickly erythrocytes (red blood cells) settle in serum, with a faster rate indicating a general, underlying inflammatory process is occurring. The technique is simple, inexpensive, and can be performed in the field without specialized equipment. We took advantage of a population of African buffalo (*Syncerus caffer*), well studied from June 2014 to May 2017, to understand the utility of ESR in an important wildlife species. When ESR was compared with other markers of immunity in African buffalo, it correlated to known measures of inflammation. We found that a faster ESR was significantly positively correlated with increased total globulin levels and significantly negatively correlated with increased red blood cell count and albumin levels. We then evaluated if ESR correlated to the incidence of five respiratory pathogens and infection with two tick-borne pathogens in African buffalo. Our results suggest that elevated ESR is associated with the incidence of bovine viral diarrhea virus infection, parainfluenza virus, and *Mannheimia haemolytica* infections as well as concurrent *Anaplasma marginale* and *Anaplasma centrale* coinfection. These findings suggest that ESR is a useful field test as an inflammatory marker in individuals and herds, helping us better monitor overall health status in wild populations.

Key words: African buffalo, disease incidence, erythrocyte sedimentation rate, inflammation, population health, *Syncerus caffer*.

INTRODUCTION

Emerging infectious diseases threaten wildlife, livestock, and human populations (Taylor et al. 2001; Merianos 2007; Cunningham et al. 2017). Many of these emerging diseases result from spillover of wildlife pathogens (Jones et al. 2008); therefore, monitoring wildlife disease has widespread benefits for multiple dimensions of ecosystem and public health. To monitor for these diseases more effectively, testing methods need to be developed and used that are 1) not exclusive to domestic animals, and 2) neither pathogen- nor host-specific.

Detecting specific diseases can be costly, time-consuming, and complex, often requiring specialized equipment and technicians. Non-specific markers of inflammation (NSMI) provide a method of detecting inflammatory status without the need for highly specialized tests. Changes in NSMI could be a useful screening tool for ongoing inflammatory

TABLE 1. Interactions between biologic components (including acute-phase proteins [APPs]) known to influence erythrocyte sedimentation rate (ESR). A greater effect is indicated by an increased number of arrows (e.g., ↑ represents a mild increase in ESR while ↑↑↑ represents a marked increase in ESR). A mixed response is given by ↓↑ whereby ESR could increase, decrease, or show no change. Areas marked — are not likely or impossible to occur (e.g., a simultaneous anemia and polycythemia).

	Within reference range	Anemia[a]	Polycythemia[a]	Increased positive APPs[b]	Increased negative APPs[b]
Within reference range[c]	No change	↑	↓	↑	↓
Anemia	↑	—	—	↑↑	
Polycythemia	↓	—	—	↓↑	↓↓
Increased positive APPs	↑	↑↑	↓↑	—	—
Increased negative APPs	↓	↓↑	↓↓	—	—

[a] Sox and Liang (1986).

[b] Lewis et al. (2006).

[c] Within reference range = all or all other components are within their reference ranges.

processes, allowing for more accurate application of pathogen-specific tests for affected individuals. Previous work by Glidden et al. (2018) in African buffalo (*Syncerus caffer*) demonstrated the utility of haptoglobin as an NSMI in this species; however, haptoglobin detection requires species-specific reagents and specialized equipment, limiting its feasibility in certain contexts.

The erythrocyte sedimentation rate (ESR) offers the potential for similar diagnostic utility to haptoglobin with the added benefit of being easy to perform in nonlaboratory settings. An inflammatory hematologic measure, ESR has been used since 1918 in the detection and monitoring of disease (Sox and Liang 1986). To obtain ESR from a sample requires only a glass tube, anticoagulant, and a whole-blood sample (Sox and Liang 1986). Variations in the ESR testing protocol have emerged to make the test even more efficient (Tishkowski and Gupta 2020) and additional equipment is not necessary. Regardless of method, ESR measures how quickly erythrocytes (red blood cells) in a blood sample tube will sediment through plasma due to gravity (Sox and Liang 1986). Increased ESR (faster sedimentation of erythrocytes) occurs in some inflammatory conditions due to electrostatic forces aggregating erythrocytes into what are called "rouleaux" (Sox and Liang 1986). Many features of the inflammatory cascade are remarkably well conserved across species. In nearly every instance of inflammation, the host's cells recognize a noxious stimulus and, through various signaling cascades, inflammatory proteins known as positive acute-phase proteins (APPs) are upregulated and negative APPs are downregulated (Janeway and Medzhitov 2002; Jain et al. 2011; Chen et al. 2017). Various products of the inflammatory cascade interact with erythrocytes differently, causing an increased or decreased ESR, with positive APPs tending to increase ESR and negative APPs tending to decrease ESR (Table 1). Other hematologic factors, such as polycythemia and hemoglobinopathies, also decrease ESR by hindering rouleaux formation (Tishkowski and Gupta 2020; Table 1).

Additionally, ESR is inexpensive, costing between US$5.00 and US$10.00 per assay in human medicine (Assasi et al. 2015), so it is a relatively cost-effective diagnostic test to detect nonspecific inflammation (Sox and Liang1986; Pääkkönen et al. 2010; Johnstone et al. 2015). In other species, ESR is even less expensive, with the average assay costing less than US$1.00 per sample in our study, for example.

Unfortunately, data on ESR as a marker of inflammation in animal species is limited and not yet fully understood. For example, ESR is known to increase in domestic dogs and cats during an inflammatory process, but in cattle and horses, ESR decreases (Johnstone et al. 2015). Due to this variability, ESR is not used

in isolation but as part of a testing protocol for initial assessment of the patient's overall health. In wildlife species, ESR has shown success in monitoring wild Eastern (*Terrapene carolina carolina*) and ornate (*Terrapene ornata ornata*) box turtle population health (Adamovicz et al. 2020) as well as gopher tortoise (*Gopherus polyphemus*) individual health (Rosenberg et al. 2018), but its value has yet to be determined in other mammalian species.

We used data from a multiyear observational study of African buffalo (*Syncerus caffer*) immunity and disease dynamics to investigate the utility of ESR in this species. African buffalo are a reservoir for several diseases that spill over into local domestic cattle herds (Kock et al. 1999; Worthington and Bigalke 2001; Musoke et al. 2015; Sisson et al. 2017); therefore, the potential economic effect on livestock makes the monitoring of buffalo health a priority. We investigated if 1) ESR correlates with markers of inflammation in African buffalo, and 2) ESR correlates with incidence or infection of respiratory and tick-borne pathogens in this species. Fibrinogen and total globulin concentrations are two categories of positive APPs most recognized for affecting ESR in people (Meyers et al. 1953), and Glidden et al. (2018) explored the utility of haptoglobin, interferon-gamma (IFNγ), and tumor necrosis factor-alpha (TNFα) as useful NSMI in African buffalo. Given these findings, the generalizability of the inflammatory cascade, and the utility of ESR as a broad indicator of inflammatory status in people, we hypothesized that ESR would positively correlate with these markers of inflammation and disease incidence in African buffalo.

MATERIALS AND METHODS

African buffalo sampled for this study were from a herd captured in northern Kruger National Park (KNP) during the early 2000s and moved to an enclosure in central KNP (Couch et al. 2017). The 900-ha enclosure included between 50–65 buffalo at any given time, due to births and deaths, as well as other herbivores and small mammalian predators typical of the ecosystem. Large herbivores such as rhinoceros (*Diceros bicornis*) and elephants (*Loxodonta africana*), and large predators such as lions (*Panthera leo*) and leopards (*Panthera pardus*), were absent. Water was available to the buffalo at a seasonal pond and a man-made water reservoir. Buffalo grazed and bred as they would in the wild; however, in the extreme dry seasons, grass and alfalfa hay were provided as supplementary feed.

Individual buffalo of mixed age and sex were sampled every 2–3 mo between June 2014 and May 2017 as part of a longitudinal study on disease dynamics in buffalo. This study has been previously described in greater detail in several published articles (Ezenwa and Jolles 2015; Couch et al. 2017, 2021; Glidden et al. 2018, 2021; Combrink et al. 2020). Our study included 532 blood samples collected from 142 individuals during 13 capture events; subsets of these samples were used for sections of our study.

Sample collection

Animals were herded into a capture corral, separated into groups of 4–10 animals, and sedated for data collection. Sedation was achieved with 7–10 μg etorphine hydrochloride and 0.04–0.07 mg azaperone (Wildlife Pharmaceuticals, White River, South Africa) per kilogram body weight for each buffalo. Buffalo that evaded corral capture were darted individually from a helicopter. Sex was determined visually, and age was determined by a combination of incisor wear and tooth emergence for animals older than 2.5 yr and via body size and horn growth in younger calves (Jolles et al. 2005). Body condition was determined by assigning a score from 1–5 based on manually palpating the ribs, hips spine, and tail base; average score was used in all analyses (Ezenwa et al. 2009). At each capture, blood was collected via jugular venipuncture with an 18-ga needle directly into several blood tubes. For red blood cell (RBC) and hematocrit levels, blood was collected into 10-mL ethylenediaminetetraacetic acid Vacutainer® tubes (Becton Dickinson, Johannesburg, South Africa). For serum biochemistry panels, coagulated whole blood was collected into Vacutainer tubes without additives (Becton Dickinson). For ESR, anticoagulated whole blood was collected in sodium-citrate Vacuette® ESR tubes (Greiner Bio-One International, Kremsmunster, Austria). Blood tubes were stored on ice for transport back to the laboratory (Glidden et al. 2018). Animals were released from the capture corral 1–5 d after capture. All animal work for this study was approved by the Institutional Animal Care and Use Committee at Oregon State University, ACUP number 4478, and at KNP.

Laboratory methods

Hematology, serum biochemistry panel, and ESR protocol: At the laboratory (approximately 6–8 h postcollection), ESR was measured using the Westergren method (ICSH 1973). After 1 h, the distance between the settled erythrocytes and the top of the supernatant plasma was quantified and reported as the ESR in mm/h. Hematologic analysis was performed on an automated impedance counter (ABC vet, SCIL Animal Care Company, Viernheim, Germany) maintained and calibrated annually by Veterinary Wildlife Services in KNP (Beechler et al. 2009, 2012). For serum biochemistry panels, coagulated blood was centrifuged at 5,000 × G for 10 min. Serum was collected into sterile microcentrifuge tubes and stored at –8 C. Frozen serum samples were then thawed and stored at 4 C for 1–3 d before analysis using an Abaxis Vetscan VS2 chemistry analyzer (Abaxis Inc., Union City, California, USA) and the large animal profile (Abaxis) maintained by Veterinary Wildlife Services in KNP. Parameters measured are outlined in Couch et al. (2017) and are stable during transport and storage as described; however, our study included only albumin, total globulins, and fibrinogen.

Disease incidence and NSMI protocol: Antibodies against five respiratory pathogens were measured in serum samples using sandwich enzyme-linked immunosorbent assays (ELISA): adenovirus-3 (Ad-3), parainfluenza virus-3 (Pi-3), bovine herpes virus (BHV), *Mannheimia haemolytica* (MH: all measured with Bio-X IPAMM pentavalent kit, Bio-X Diagnostics, Rochefort, Belgium). Samples were considered positive if antibody titers exceeded threshold absorbance values calculated using quality control procedures outlined in each Bio-X kit. Incidence was calculated as a binomial variable, with a "1" indicating an animal seroconverted from t_0 to t_1 (i.e., absorbance values were below threshold concentrations at t_0 but above threshold absorbance at t_1) and a "0" indicating the animal had not seroconverted. All respiratory pathogen ELISAs were run within 2 wk of capture periods as described in Glidden et al. (2018, 2021).

To isolate and characterize the tick-borne pathogens *Anaplasma marginale* (AM) and *A. centrale* (AC), DNA was extracted from whole-blood samples that were collected in ethylenediaminetetraacetic acid–coated tubes and stored at –80 C. *Anaplasma* spp.-specific genes were amplified using nested or conventional PCR and specific primers as described in Sisson et al. (2017). Negative and positive controls were included in each PCR test (Sisson et al. 2017).

Cytokine assays were run using incubated plasma samples and were measured via sandwich ELISA per manufacturer's instructions (Haptoglobin: Life Diagnostics 2410; TNFα: Ray-Bio ELB-TNFα; IFNγ: Bio-Rad MCA5638KZZ) as described (Glidden et al. 2018) within 1 mo of collection. Due to the distribution of haptoglobin measurements, values were recorded as either high or low, with high haptoglobin exceeding the 90th percentile in relation to study samples only (Glidden et al. 2018).

Statistical methods

All statistical analyses were conducted in R (R Core Team 2020). We used linear mixed effects models in all situations and included animals with all relevant data in each model (Bates et al. 2015; Kuznetsova et al. 2017). To evaluate the effect of other hematologic inflammatory markers on ESR, we built a global model including animal age, sex, body condition score, RBC count, manually calculated hematocrit, albumin, total protein, haptoglobin, globulins, fibrinogen, IFNγ, and TNFα. We included 69 animals from six capture time points for a total of 302 observations. We then built a global model including known markers of inflammation that had a significant effect on ESR (albumin, globulin, RBC count) and pathogen incidence for five respiratory pathogens as covariates. We included 86 animals from 13 capture time points for a total of 532 observations. Due to low sample size, we ran a separate model for the effect of current *Anaplasma* spp. infection on ESR. This model was built in the same manner as the respiratory pathogen model, including interaction terms between *Anaplasma* spp. and ESR, but included 67 animals from two capture time points for a total of 107 observations.

To account for our repeated-measures study design, we initially included animal ID number and capture number as random intercepts in all models. In models evaluating the effect of pathogen incidence or infection on ESR, we found that variation due to animal ID was nonsignificant and only ran the models with capture number as a random effect. For model selection, we calculated the Akaike information criterion with small-sample correction (AICc; Hurvich and Tsai 1989) for all combinations of variables that included significant markers of inflammation as covariates. For our final model, we selected the most parsimonious model within two AICc units (Hurvich and Tsai 1989) of the model with the lowest AICc. We then calculated marginal and conditional R^2 to further evaluate model fit. Full details on statistical analyses, including checking model assumption, checking for influential data, packages used, and supplementary models are available in the Supplemental Material.

TABLE 2. Final linear mixed model for erythrocyte sedimentation rate (ESR) and known markers of inflammation in African buffalo (*Syncerus caffer*), including capture period and animal identity as random effects. Sex, interferon-gamma (IFNγ), body condition score (BCS), haptoglobin, and fibrinogen levels were not significantly correlated to ESR ($P>0.05$). Red blood cell (RBC) and albumin levels were significantly negatively correlated with ESR ($P<0.05$), and globulin levels significantly positively correlated with ESR ($P<0.05$; marginal R^2=0.091 and conditional R^2=0.252).

Predictors	Estimates	SE	*F* statistic	*P*[a]
(Intercept)	36.58	5.23	6.99	<0.001
Globulin	2.69	1.31	2.05	**0.040**
RBC count	−3.70	1.36	−2.71	**0.007**
Albumin	−3.73	1.55	−2.41	**0.016**
Fibrinogen	2.18	1.31	1.66	0.097
IFNγ	−2.15	1.45	−1.48	0.139
Haptoglobin	3.60	4.11	0.88	0.381
BCS	1.76	1.90	0.93	0.354
Sex	4.33	2.71	1.60	0.261

[a] Boldface values indicate significant parameters.

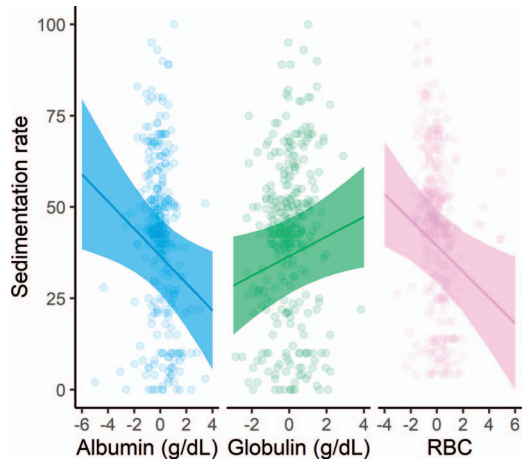

FIGURE 1. Final model output of erythrocyte sedimentation rate (ESR) vs. inflammatory markers for African buffalo (*Syncerus caffer*) showing the median (solid line) and 95% confidence interval (ribbon) for albumin (blue), globulin (green), and red blood cell (RBC) count (pink) levels. As albumin levels increase, ESR decreases, corresponding to a slower sedimentation rate (P=0.016). Total globulin levels vs. ESR. As globulin levels increase, ESR increases, corresponding to a faster rate (P=0.040). As RBC count increases, ESR decreases, corresponding to a slower sedimentation rate (P=0.007).

RESULTS

ESR and other markers of inflammation

We tested whether ESR was correlated to other measures of inflammation (albumin, RBC count, fibrinogen, total globulins, haptoglobin, IFNγ, and TNFα) in buffalo, while accounting for sex, body condition, individual, and capture period. Our best and most parsimonious model included all variables except for TNFα, which did not improve model fit and was removed. We found ESR to be negatively correlated with RBC count (Table 2 and Fig. 1): with an increase in RBC count, ESR was slower. Similarly, we found ESR to be negatively correlated with albumin (Table 2 and Fig. 1). We found globulins to be positively correlated with ESR: as globulin levels increased, ESR was faster (Table 2 and Fig 1; marginal R^2=0.091 and conditional R^2=0.252). Mean ESR was approximately 37 mm/h across all buffalo; however, mean ESR was approximately 33 mm/h among buffalo with higher RBC count and albumin levels and 39 mm/h among buffalo with higher globulin levels (Table 2).

Disease incidence and ESR

We found that ESR significantly increased with the incidence of three solo respiratory infections: BVDV, MH, and Pi-3 (Table 3 and Fig. 2). However, ESR was marginally slower in buffalo with Ad-3 (Table 3 and Fig. 2) and significantly slower in buffalo with Ad-3 and Pi-3 coinfection or Pi-3 and BHV coinfection (Table 3 and Fig. 3; marginal R^2=0.19 and conditional R^2=0.36). The mean ESR of all buffalo with respiratory pathogens was 40.14 mm/h (Table 3). Buffalo that seroconverted for BVDV between capture periods had a mean ESR of 55.31 mm/h, those that seroconverted for MH had a mean ESR of 49.67 mm/h, and those that seroconverted for Pi-3 had a mean ESR of 54.54 mm/h (Table 3). In buffalo that seroconverted for both Ad-3 and Pi-3, the mean ESR was 10.88 mm/h and in those that serocon-

TABLE 3. Final linear mixed model for erythrocyte sedimentation rate (ESR) and five respiratory pathogens in African buffalo (*Syncerus caffer*) including capture period as a random effect. Albumin, globulin, and red blood cell (RBC) levels were included as they have been previously shown to influence ESR. Bovine viral diarrhea virus (BVDV), *Mannheimia haemolytica* (MH), and parainfluenza virus-3 (Pi-3) infections were all significantly positively correlated with ESR ($P<0.05$). Adenovirus-3 (Ad-3) and Pi-3 co-infection, as well as Pi-3 and bovine herpes virus (BHV) co-infection, were significantly negatively correlated with ESR ($P>0.05$). Ad-3 was only suggestive of a negative correlation with ESR ($P=.056282$; marginal $R^2=0.19$ and conditional $R^2=0.36$).

Predictors	Estimates	SE	*F* statistic	*P*[a]
(Intercept)	40.14	4.26	9.43	<0.001
BVDV	15.17	4.31	3.52	**<0.001**
Pi-3	14.40	4.48	3.21	**0.001**
MH	9.53	4.62	2.06	**0.039**
Ad-3:Pi-3	−29.26	11.38	−2.57	**0.010**
Pi-3:BHV	−30.22	12.14	−2.49	**0.013**
BHV	−6.31	5.96	−1.06	0.289
Ad-3	−10.58	5.86	−1.81	0.071
Albumin	−5.71	2.89	−1.98	**0.048**
Globulin	2.61	2.71	0.96	0.337
RBC count	−4.63	2.41	−1.92	0.055

[a] Boldface values indicate significant parameters.

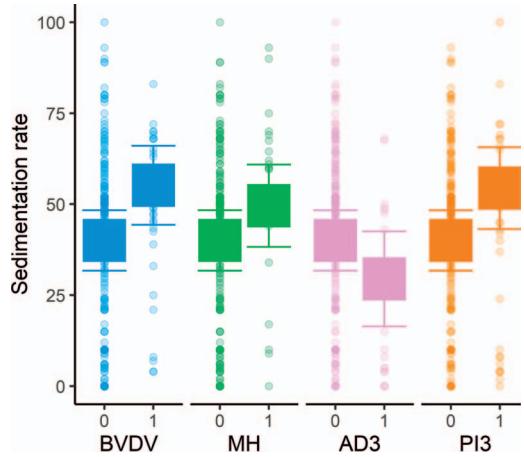

FIGURE 2. Final model output of erythrocyte sedimentation rate (ESR) vs. disease incidence for bovine viral diarrhea virus (BVDV; blue), *Mannheimia* haemolytica (MH; green), Adenovirus-3 (Ad-3; pink), and parainfluenza virus-3 (Pi-3; orange) in African buffalo (*Syncerus caffer*). Model predictions for mean ESR (confidence interval=95%) overlay individual data points for each graph. Seroconversion was used as proxy for disease incidence, where no disease incidence = 0, and disease incidence = 1. The ESR significantly increased with BVDV, MH, and Pi-3 incidence and marginally significantly decreased with Ad-3 incidence.

verted for both Pi-3 and BHV the mean ESR was 9.92 mm/h (Table 3).

Additionally, ESR significantly increased in buffalo coinfected with the two tick-borne pathogens, AM and AC (Table 4 and Fig. 4), but significantly decreased with AM infection alone (Table 4 and Fig. 4; marginal $R^2=0.086$ and conditional $R^2=0.086$). The mean ESR among buffalo infected with one or both tick-borne pathogens was 39.16 mm/h (Table 4). Among buffalo infected with only AM, the mean ESR was 24.77 mm/h, and among buffalo coinfected with AM and AC, the mean ESR was 65.8 mm/h (Table 4).

DISCUSSION

Measurements of generalized inflammation, such as ESR, are critical not only for disease detection but also for monitoring population health (Ryser-Degiorgis 2013). Our findings that ESR was correlated to other measures of inflammation in African buffalo, and that it changed with disease incidence or ongoing infections, suggest that ESR is a promising tool for monitoring wildlife health that is relatively inexpensive and requires little to no specialized equipment or training.

Finding that ESR was positively correlated with total globulins in African buffalo is interesting. Total globulin levels can be broken down into alpha, beta, and gamma globulins with gamma globulins (i.e., antibodies) having a significant effect on ESR in people (Meyers et al. 1953). The time course for increased antibody production (hypergammaglobulinemia) in response to an infection varies between species and infectious agents. In cattle, various pathogens can lead to hypergammaglobulinemia, occurring anywhere from 4–120 d postexposure (Goldman and Pipano 1978; Pega et al. 2013). As our data were collected from a managed, free-ranging herd, we did not know when exactly

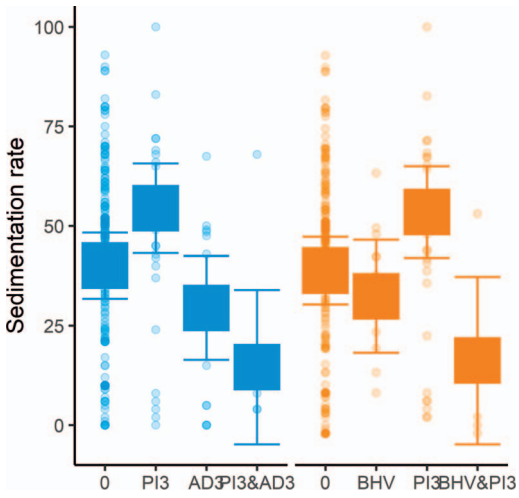

FIGURE 3. Final model output of erythrocyte sedimentation rate (ESR) vs. disease incidence for African buffalo (*Syncerus caffer*) coinfected with adenovirus-3 and parainfluenza virus-3 (Ad-3, Pi-3; blue), as well as those coinfected with bovine herpes virus (BHV) and Pi-3 (orange). Model predictions for mean ESR (confidence interval=95%) overlay individual data points for each graph. In this model, seroconversion was used as a proxy for disease incidence. The ESR for infection with singular pathogens is shown adjacent to ESR in those with coinfections. ESR significantly decreased with Pi-3 and Ad-3 coinfection, as well as with BHV and Pi-3 coinfection.

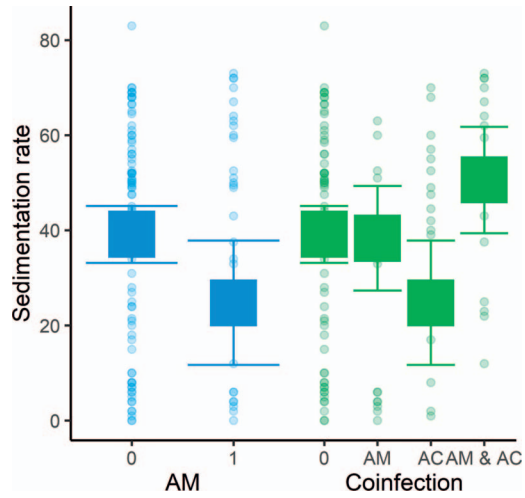

FIGURE 4. Erythrocyte sedimentation rate (ESR) vs. disease incidence for *Anaplasma marginale* (AM) infection (blue) and AM and *Anaplasma centrale* (AC) coinfection (green) in African buffalo (*Syncerus caffer*). In this model, absence of infection = 0 and true infection = 1. The ESR marginally significantly decreased with AM infection; however, ESR significantly increased with AM and AC coinfection.

TABLE 4. Final linear mixed model for erythrocyte sedimentation rate (ESR) and two tick-borne pathogens, *Anaplasma marginale* (AM) and *A. centrale* (AC) in African buffalo (*Syncerus caffer*), including capture period as a random effect. Red blood cell (RBC) count, and albumin and globulin levels were included in the model as they have previously been shown to influence ESR. Infection with AM was significantly negatively correlated with ESR ($P<0.05$); however, AM and AC coinfection was significantly positively correlated with ESR ($P<0.05$; marginal $R^2=0.086$ and conditional $R^2=0.086$).

Predictors	Estimates	SE	*F* statistic	*P*[a]
(Intercept)	39.16	3.04	12.87	<0.001
AM	−14.39	7.33	−1.96	**0.049**
AC	−0.84	6.36	−0.13	0.895
AM:AC	26.64	10.72	2.49	**0.013**
RBC count	0.50	4.97	0.10	0.919
Albumin	2.06	5.03	0.41	0.682
Globulin	4.48	4.71	0.95	0.341

[a] Boldface values indicate significant parameters.

an individual became infected with a pathogen; however, increases in globulin levels due to infection probably explain the significant positive correlation between ESR and total globulin levels.

We also found ESR to be significantly negatively correlated with RBC count in African buffalo. Sox and Liang (1986) stated that a low RBC count corresponded to an elevated ESR in people. Tishowski and Gupta (2020) also found that high RBC count was associated with a decreased ESR, as seen in our model. We also found ESR to be significantly negatively correlated to albumin levels (Fig. 1). Lewis et al. (2006) found that albumin inhibits the process of erythrocyte aggregation in humans, leading to a decreased ESR. A similar process may be occurring in buffalo, but future work would be needed to clarify the mechanism. Despite lack of knowledge of mechanisms, because ESR did correlate with changes in RBC count, albumin, and globulin levels, it may be a useful replacement for these values. Serum biochemistry analysis and complete blood counts can cost upward of

US$50 per animal (University of Miami 2020); therefore, ESR could offer a more cost-effective method of determining inflammatory status in wildlife species.

Interestingly, we did not find a significant correlation between ESR and fibrinogen, haptoglobin, or IFNγ levels. These molecular markers are all positive APPs, which have been previously reported to affect ESR (Sox and Liang 1986; Lewis et al. 2006; Johnstone et al. 2015; Tishowski and Gupta 2020). Fibrinogen is known to increase in several inflammatory conditions in cattle (Hirvonen et al. 1996; Cheryk et al. 1998; Hirvonen and Pyorala 1998); however, we found the range of fibrinogen values to be narrow and unhelpful in discriminating inflammatory responses between individual buffalo. With viral and bacterial infections, IFNγ is upregulated and promotes production of cytokines that stimulate the immune system. As such, IFNγ upregulation happens within minutes to hours of infection (Schoenborn and Wilson 2007) and declines quickly (within 1 wk) after initial infection (Glidden et al. 2018). We had no way to calculate how long after infection we were collecting blood samples; therefore, it is possible that samples were taken either before or after peak IFNγ secretion in response to pathogen exposure. Glidden et al. (2018) concluded that haptoglobin remained elevated for an average of 21 d in African buffalo naturally exposed to Pi-3 and *Mycoplasma bovis* as well as those experimentally infected with foot and mouth disease virus. We did not have sufficient ESR values from *M. bovis*-positive or foot and mouth disease virus-positive animals to investigate how these pathogens may correlate with ESR. It is possible that haptoglobin behaves differently in buffalo infected with these pathogens compared to pathogens included in this model. Future research could investigate ESR and positive APP levels at specific time points over the course of known or experimentally induced infections in African buffalo.

After establishing that ESR correlates with some inflammatory markers (globulins, albumin, and RBC count) in African buffalo, we then explored whether ESR would correlate with disease incidence in this species. We found that an increase in ESR correlated with buffalo seropositive for BVDV, MH, or Pi-3 (Table 3 and Fig. 2), all pathogens that lead to changes in white blood cell count and cause inflammation (Von Donkersgoed et al. 1993; Lally et al. 1999; Müller-Doblies et al. 2004; Rice et al. 2007; Ridpath et al. 2007; Ellis 2010; Lanyon et al. 2014). It is likely that inflammatory processes due to active infection(s), as well as the up- or downregulation of other pathways in response to these pathogens, led to the observed changes in ESR in our study (Table 1). Studies further investigating the relationship between these pathogens and their direct effects on ESR need to be conducted to understand the mechanisms driving these pathways.

Additionally, coinfection with AM and AC was significantly positively correlated with ESR (Table 4 and Fig. 4). These two pathogens are both obligate intracellular pathogens of erythrocytes. The alteration in ESR seen with AM and AC could be due to alterations in RBC morphology or membrane charge as well as to other changes in inflammatory processes during infection. Unfortunately, anaplasmosis in African buffalo is not fully understood. In South Africa, coinfection with multiple tick-borne pathogens is relatively common in several animal species (De Waal 2000). Recent work by Henrichs et al. (2016) and Sisson et al. (2017) investigating *Anaplasma* spp. in African buffalo has also shown that coinfections with AM and AC are common. It is possible that either coinfected animals or those repeatedly infected with the same pathogen mounted a dramatically increased immune response compared to singly infected individuals; however, further studies are needed to understand the physiologic effects of *Anaplasma* spp. coinfection on ESR in African buffalo.

We found that ESR was marginally negatively correlated with the incidence of Ad-3 infection and AM infection (Tables 3, 4 and Figs. 2, 4). Buffalo that were coinfected with Ad-3 and Pi-3, as well as BHV and Pi-3, also had a negative correlation with ESR (Table 3 and Fig. 3). It is possible that coinfected

buffalo were somewhat immunocompromised (Rosenquist and Dobson 1974), hence why they were infected with multiple pathogens. This immunosuppression might have prevented animals from activating biologic processes that alter ESR (Table 1). *Anaplasma marginale* is known to evade the host immune system (Han 2008), perhaps leading to the marginal correlation with a decreased ESR found in our study.

We did not find ESR to correlate with BHV incidence alone; however, BHV, like other herpesviruses, goes through periods of activation and latency (Nandi et al. 2009). Although these animals were seropositive for infection, there may not have been a large enough inflammatory response during sampling to affect ESR.

There are multiple combinations of processes that may alter ESR (Table 1), making it difficult to assess which specific physiologic pathway is driving the changes observed in our study. It is important to note that although some infections correlated to an increase in ESR, while others correlated to a decrease in ESR, infections did lead to an abnormal measurement. When evaluating other hematologic measurements, such as RBC count or albumin levels, both high and low values can indicate pathology, not just those that are increased. Therefore, a decreased ESR may be just as indicative of pathology as is an increased value, making ESR a good potential candidate for an NSMI in African buffalo.

There are limitations to the use of ESR, however. Tilted tubes, vibrations, oscillating room temperatures, and improper filling of the glass tube can all affect the final value (Tishowski and Gupta 2020). In humans, markedly increased (>100 mm/h) ESR values are highly specific for significant underlying disease (Tishkowski and Gupta 2020), but ESR probably suffers from decreased specificity at lower values. For these reasons, ESR is seldom used in isolation, and future studies to validate the use of ESR as an NSMI should investigate ESR patterns with the specific disease of concern. Additionally, if we want to better monitor population health in a variety of both captive and managed wildlife, we must evaluate ESR in a range of species, creating reference ranges for each, the same way we have done for humans and domestic animals.

We found that ESR has potential utility as a broad indicator of inflammatory status in African buffalo. It is easily performed in the field and requires minimal expertise, training, and equipment. The assay is rapid, cost effective, and takes advantage of well-documented inflammatory processes that are relatively conserved across species. We found that ESR changed with RBC count, albumin, and globulin levels in expected ways, as well as with infection status. It is therefore a promising metric of inflammatory status in African buffalo and could be used in conjunction with other indicators to gain a more complete picture of population health.

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SUPPLEMENTARY MATERIAL

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