Unusual blood profiles in the endemic Kagu of New Caledonia are not physiological

Roman Gula¹ · Henri Bloc² · Thomas Duval³ · Jörn Theuerkauf¹

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Abstract

Previous research reported a unique haematological profile in the flightless Kagu (*Rhynochetos jubatus*). To verify this, we analysed blood of eight wild and four captive Kagu. The haematological profiles of our captive birds resembled those of the previously studied captive Kagu. Haemoglobin concentration, mean corpuscular haemoglobin concentration and mean corpuscular haemoglobin by far exceeded the highest levels recorded for birds. Conversely, the blood profile in wild Kagu fit in the range of other birds. We conclude that blood profiles of captive Kagu indicate a pathology, and that Kagu do not have fundamentally different blood characteristic than other endothermic vertebrates.

Keywords Flightless bird · Haemoglobin · Heavy metal · Captivity

Zusammenfassung

Ungewöhnliche Blutbilder des endemischen Kagu aus Neukaledonien sind nicht physiologisch.

Eine ältere Studie berichtet über ein außergewöhnliches Blutbild beim flugunfähigen Kagu (*Rhynochetos jubatus*). Um dies zu überprüfen, analysierten wir Blut von acht wildlebenden und vier in Gefangenschaft lebenden Kagus. Wir kamen bei den Blutbildern der in Gefangenschaft lebenden Kagus zu ähnlichen Ergebnissen wie die vorherige Studie. Hämoglobinkonzentration (Hb), mittlere Hämoglobinkonzentration der roten Blutkörperchen (MCHC) und mittlerer Hämoglobingehalt eines roten Blutkörperchens (MCH) übertrafen bei weitem die höchsten bei Vogelarten gemessenen Werte. Das Blutbild wildlebender Kagus dagegen entsprach der Variationsbreite anderer Vogelarten. Wir schließen daraus, dass Blutprofile von in Gefangenschaft lebenden Kagus auf eine Pathologie hindeuten und dass Kagus kein grundlegend anderes Blutbild haben als andere warmblütige Wirbeltiere.

Introduction

In vertebrates, red blood cell size and numbers widely vary among species, but the mean haemoglobin concentration is relatively constant in endothermic vertebrates at around 10–22 g/dl (Hawkey et al. 1991, see Table 1). However, Vassart (1988) reported an about 50% higher haemoglobin concentration and nearly three times more corpuscular haemoglobin in the flightless Kagu (*Rhynochetos jubatus*), an endemic bird of New Caledonia, than the highest values in any other bird species. Although the study of Vassart (1988) was based on blood samples taken from only three captive birds, these blood characteristics were so unique among birds, and even among vertebrates, that the information was widely disseminated (e.g. Hunt 1996, <https://en.wikipedia.org/wiki/Kagu>). At the same time, Kagu can withstand very high concentrations of heavy metals in their

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✉ Roman Gula
rgula@miiz.eu
Jörn Theuerkauf
jtheuer@miiz.eu

¹ Museum and Institute of Zoology, Polish Academy of Sciences, Warsaw, Poland

² Conservation Research New Caledonia, New Caledonia and Syndicat Mixte Des Grandes Fougères, Farino, New Caledonia

³ Hémisphères, Poindimié, New Caledonia

Table 1 Blood parameters (averages with 95% confidence intervals) of 15 Kagu compared to the range observed in reptiles, birds and mammals (Hawkey et al. 1991)

Source	Status	Sex	Age (years)	HCT (%)	RBC ($10^{12}/l$)	HGB (g/dl)	MCV (fl)	MCHC (g/dl)	MCH (pg/cell)	WBC ($10^9/l$)	PLT ($10^{11}/l$)
Vassart (1988)	Captive	F	6	37	1.3	33.2	290	91.0	263	–	–
Vassart (1988)	Captive	M	3	41	1.1	37.8	373	92.2	344	–	–
Vassart (1988)	Captive	M	3	39	1.1	31.0	355	79.5	282	–	–
This study	Captive	F	22	40	1.7	39.2 ^a	235	98.0	231	16.8	b
This study	Captive	M	1.5	48	1.8	29.2	270	60.8	164	20.0	0.2
This study	Captive	M	1.5	42	1.8	29.0	240	69.0	166	26.0	0.2
This study	Captive	F	1.5	40	1.8	29.1	225	72.8	164	18.8	0.1
This study	Wild	F	> 6	51	2.3	19.8	222	38.8	86	–	–
This study	Wild	F	> 2	45	2.2	17.9	207	39.8	83	–	–
This study	Wild	F	> 2	50	2.1	21.2	245	42.4	104	–	–
This study	Wild	M	> 11	59	2.9	19.0	202	32.2	65	31.6	b
This study	Wild	M	6	57	2.9	20.1	197	35.3	70	35.2	0.1
This study	Wild	M	5	46	2.3	19.5	203	42.4	86	51.2	0.1
This study	Wild	M	> 4	48	2.4	21.6	201	45.0	90	32.4	0.1
This study	Wild	M	> 2	49	1.8	18.9	266	38.6	103	–	–
Average Vassart				39±3	1.2±0.1	34.0±3.9	339±49	87.5±7.9	296±48		
Average captive this study				43±4	1.8±0.1	31.6±4.9	242±19	75.2±15.7	181±32	20.4±3.9	0.2±0.1
Average wild				51±3	2.4±0.3	19.8±0.8	218±17	39.3±2.9	86±10	37.6±9.0	0.1±0.0
Range reptiles				15–39	0.2–1.6	5.1–12.0	214–707	22.5–39.3	60–271		
Range birds				28–59	1.5–5.0	10.3–19.3	105–286	27.1–40.2	31–108		
Range mammals				28–58	1.9–20.1	10.1–22.1	19–156	27.3–45.9	7–61		

HCT haematocrit (packed cell volume), *RBC* red blood cell count, *HGB* haemoglobin concentration, *MCV* mean corpuscular volume, *MCHC* mean corpuscular haemoglobin concentration, *MCH* mean corpuscular haemoglobin, *WBC* white blood cell (leucocyte) count, *PLT* thrombocyte (platelet) count

^aValue possibly too high because of lipemic blood

^bCount impossible or unreliable

diet and have especially high concentration of chromium in their feathers (Theuerkauf et al. 2015, 2017). The unique blood profile could, therefore, have been an adaptation to the particular environmental conditions in New Caledonia, where one-third of the land surface is covered with ultra-mafic soil rich in heavy metals (Becquer et al. 2003). High levels of haemoglobin packed in relatively few, large cells may theoretically allow Kagu to minimize harmful effects of some elements (like Cr^{+4}) on haemoglobin (Tchounwou et al. 2012). As Vassart (1988) studied exclusively captive Kagu, we analysed the blood parameters of wild and captive Kagu to verify if the unique blood profile is physiological and thus a potential adaptation.

Methods

In December 2017 and February 2018, we collected blood samples from eight wild adult Kagu (three females and five males) living in the Parc des Grandes Fougères (mean annual temperature 20 °C) at altitudes ranging from 300 to 500 m a.s.l. (21° 30–39' S, 165° 39–50' E). As the sampled Kagu belonged to a population studied since 2011 by radio tracking and molecular genetics (Theuerkauf et al. 2018), we knew their sex and age and could capture them at night roost. Additionally, in January 2019, we collected blood samples from four captive Kagu (two males and two females) of the Parc Zoologique et Forestier in Nouméa (about 50 m a.s.l., average annual temperature 23.5 °C). We took blood samples (0.5 ml, which represents less than 0.1% of body mass and less than 1% of the blood volume of a Kagu) from the medial metatarsal vein with a syringe and transferred the blood to EDTA microtubes. As we took blood samples of wild Kagu at night, we immediately stored them at about 4 °C to avoid morphological and fragility changes in blood cells (Antwi-Baffour 2013). The next day, about 12 h after collection, we brought the sample tubes for analysis to the veterinarian laboratory of New Caledonia (service des Laboratoires officiels vétérinaires, agroalimentaires et phytosanitaires de la Nouvelle-Calédonie, Direction des Affaires Vétérinaires, Alimentaires et Rurales, Païta, New Caledonia), which counted red, white blood cells and thrombocytes (RBC, WBC, PLT), and which measured haemoglobin concentration (HGB) and haematocrit (HCT) using standard procedures (manual count for RBC and WBC, colorimetry for HGB, micro centrifugation for HCT). As we could capture captive Kagu in the daytime, we stored the sample tubes at about 4 °C for only 3 h before analysis (same procedures in the same laboratory as samples of wild Kagu). We calculated mean corpuscular volume ($\text{MCV} = 1000\text{HCT}/\text{RBC}$), mean corpuscular haemoglobin concentration ($\text{MCHC} = 10\text{HGB}/\text{HCT}$) and mean corpuscular haemoglobin ($\text{MCH} = 10\text{HGB}/\text{HCT}$).

Results

Captive Kagu in our study had low red blood cell counts and high haemoglobin concentration (Table 1), but not to the extent of Kagu studied by Vassart (1988). This led to very high calculated values of mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration. Captive Kagu also had substantially lower leucocyte counts than wild individuals (Table 1). In wild Kagu, red blood cell counts were higher, whereas haemoglobin levels (HGB, MCHC, MCH) were lower than in captive birds (Table 1). Mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration in wild Kagu were within the range of other bird species, but haemoglobin concentration was slightly higher than the highest recorded in birds (Table 1). No Kagu had haemoparasites.

Discussion

Blood profiles of captive Kagu in our study were intermediate between those of wild Kagu and captive Kagu studied by Vassart (1988) although haemoglobin concentration, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration exceeded (sometimes by far) the highest values recorded for birds. Conversely, the blood profiles of wild Kagu were within the range for birds (see review in Hawkey et al. 1991 in Table 1). The unusual blood profile of captive Kagu is, therefore, not an adaptation of the species to the specific environment of New Caledonia but rather has a pathological origin. Especially, the low counts of red and white blood cells in captive birds point at an anaemia. Low red blood cell counts usually co-occur with low haemoglobin concentration (Jones et al. 2002). However, very high haemoglobin concentration combined with low red blood cell counts could be associated with a haemolytic anaemia, as haemoglobin from destroyed red blood cells would still be present in the plasma, and could be detected by analytical procedures. Although there were no indications of destroyed blood cells during the blood analyses, we cannot exclude the possibility that red blood cells deteriorated in an earlier stage and were, therefore, not detectable during the cell count. Accordingly, the observed unusually high values of mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration in captive Kagu might be artefacts and a by-product of the calculation methods.

The most common causes of macrocytosis and haemolytic anaemia are inadequate diet (especially deficiency of vitamin B12), bone marrow and liver disorders or infections (Shaw et al. 2009). The diet of Kagu kept in the

Parc Zoologique et Forestier in Nouméa mainly consists of bovine heart and could be the reason for the pathological blood pattern. The low frequency and small volume of defaecation by captive Kagu and the discoloration of their bills and legs that we observed also point at a deficiency in their diet. Further veterinary studies, however, would be necessary for a precise diagnosis of the mechanisms causing the observed blood anomaly.

High, but physiological haemoglobin concentration, haematocrit and red blood cell count are a good proxy for the condition of birds as they correlate with body mass and body fat (Minias 2015). Therefore, our results imply that wild Kagu were in a better condition than captive birds (Fair et al. 2007), probably due to more appropriate nutrient contents of the natural diet and more physical exercise associated with foraging. This also complies with the absence of haemoparasites and negligible infestation of endemic blood ectoparasites (Beugnet et al. 1995) that we observed in the studied wild population of Kagu.

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