

Isoflurane anesthesia alters complete blood count values in lilac-crowned parrots (*Amazona finschi*)

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OBJECTIVE

To evaluate the effects of a chemical restraint with isoflurane compared to a physical restraint in hemogram values in psittacines.

ANIMALS

21 adult lilac-crowned parrots (*Amazona finschi*).

METHODS

Subjects were physically restrained for blood sample collection, after this isoflurane anesthesia was administered for 30 minutes at which time a second blood sample was taken. Hemogram values were compared between both samples using a *t* test in variables with normal distribution and a Wilcoxon test in variables without normal distribution. Statistical significance was established at $P < .05$.

RESULTS

When comparing the analytes of the respective CBCs, a decrease in PCV ($P < .001$), RBC count ($P < .001$), WBC count ($P = .017$), absolute lymphocytes ($P = .024$), and total proteins ($P < .001$) was observed in the samples taken under chemical restraint. No significant differences in MCV and absolute values of heterophils, monocytes, or eosinophils were observed.

CLINICAL RELEVANCE

Isoflurane causes a decrease in some blood count values in lilac-crowned parrots under isoflurane anesthesia for 30 minutes. Although the magnitude of change could be variable between values, it is important to consider the blood collection method when interpreting the blood count in this species.

Keywords: complete blood count, isoflurane, anesthesia, psittacines, *Amazona finschi*

The *Psittaciformes* order contains some of the most popular species maintained in captivity in zoos or as companion animals. These species present diverse diseases whose diagnosis requires the use of complementary tests. Inhaled anesthesia has been recommended for short procedures like x-rays, blood sampling, and physical examinations to diminish stress and have better control of the patient.^{1,2} Isoflurane is a frequently used inhaled anesthesia

in birds because it has a quick and easy induction and recovery. It is not required to know the weight of the species because isoflurane is metabolized in the liver and eliminated through the lungs.³ However, it has been described that inhaled anesthesia with isoflurane produces effects on the CBC in different mammals such as ferrets (*Mustela putorius furo*),⁴ guinea pigs (*Cavia porcellus*),⁵ bats (*Pteropus hypomelanus* and *Artibeus jamaicensis*),^{6,7} and rats (*Rattus norvegicus*)^{8,9} and different birds such as owls (*Bubo virginianus*),¹⁰ dusky-legged guans (*Penelope obscura*),¹¹ falcons (*Falco sparverius*),¹² and eagles (*Spilornis cheela hoyi*).¹³ The reported

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findings include a decrease in PCV, WBC count, total solids, platelets, and thrombocyte count. Data on the effects of isoflurane on blood cell counts and chemistry values for the order *Psittaciformes* have been discussed previously in cockatoos,¹⁴ and results show that PCV, lymphocytes, and monocytes differ significantly compared with samples taken by manual restraint.

The Google Scholar, PubMed, Science Direct, and JSTOR databases were used to search for publications related to the topic using the words: parrot, psittacine, isoflurane, hematological effects, and CBC. No experimental studies on isoflurane alterations in CBC for lilac-crowned parrot (*Amazona finschi*) were found. This promotes extrapolation of data obtained in other species without considering that effects are variable, since protocols between studies¹⁰⁻¹³ differ in anesthesia maintenance time and in number of days for sampling. The purpose of the study was to evaluate the effects of a chemical restraint with isoflurane compared to a physical restraint in the CBCs of 21 lilac-crowned parrots (*A finschi*) in captivity. We hypothesized that in lilac-crowned Amazon under chemical contention with isoflurane, the PCV, RBCs, WBCs, thrombocytes, and total solids will be lower when compared to those CBC values obtained in physically restrained subjects. This will allow us to take into consideration the restraint method and the effects of isoflurane when interpreting blood count values in parrots.

Methods

Animals

Twenty-one adult lilac-crowned parrots (*A finschi*) older than 1 year of age, with a weight of 218 to 377 g and unidentified sex, were used in this study. These animals belong to Bosque y Zoológico de la Ciudad of Mexicali, Baja California, México. To prevent a higher anesthetic risk, subjects with signs suggesting an active pulmonary disease like nasal or eye secretions, respiratory distress, weakness, or ruffled feathers were not included in the study. These parrots were obtained from 3 different lodgings distributed in the zoo, made with a galvanized mesh measuring 5 meters high, 6 meters long, and 5 meters wide. The light cycle was natural, and the temperature range during the time the study was conducted varied between 5 and 17 °C (41 and 62 °F). Their diet consists mostly of fresh fruits (apple, pear, papaya, raspberry, strawberry, and banana), vegetables (peppers, broccoli, carrots, mushrooms, and spinach), sunflower seeds, peanuts, and Mazuri Small Bird Diet Brand pellet food. Water was offered fresh in containers that were distributed throughout the accommodation.

Experimental design

This study was reviewed and approved by the Internal Committee for the Care and Use of Animals from the Facultad de Medicina Veterinaria y Zootecnia of Universidad Nacional Autónoma de México. The protocol number was 096.

The birds were fasted for 2 hours before handling. Physical restraint was performed with a towel, and the temperature, heart, and respiratory rate were evaluated. If the bird showed signs of weakness, hyperthermia, tachypnea or escaped from the towel or cage when handlers were trying to catch it, it was placed back in the cage and tested the next day. Blood samples were collected by venipuncture of the jugular vein using a 1-mL syringe with a 25-G needle. A maximum of 0.5 mL and a minimum of 0.3 mL of blood were collected between each sample. Hemostasis was performed with digital pressure with a cotton swab on the punctured site. Immediately after the sample was obtained, induction with isoflurane gas (Sofloran Vet; Pisa Agropecuaria Inc) was started via inhalation at 5% at 1 L/min of oxygen through a mask. The approximate restraint time for each bird before anesthesia was between 5 and 10 minutes.

The type of anesthetic circuit used was closed with a calibrated isoflurane vaporizer (Isoflurane Vaporizer 19.1; Drägerwerk AG & Co). Once the subject did not respond to tactile stimuli and did not have positioning or palpebral reflex, the anesthesia was reduced by 0.5% every 30 seconds until reaching a maintenance percentage of 1.5% and 2% to subsequently place a 2.0-uncuffed endotracheal tube (Sensi Medical).

The birds were placed on a thermal mat with an approximate temperature of 27 to 30 °C (80.6 to 86 °F; Thermal Plate; Petmmal Inc). During maintenance, every 5 minutes, cardiac and respiratory frequencies were monitored by auscultation using a stethoscope and cloacal temperature through a digital thermometer. The average body temperature, heart rate, and respiratory rates were 40 °C, 261 bpm, and 61 bpm, respectively.

After 30 minutes of maintenance, another blood sample was taken. Isoflurane administration was subsequently discontinued, and the bird was allowed to recover with 100% oxygen. When completely recovered, the birds were kept under 24-hour observation and then released into their original enclosure.

Sample storage and processing

Blood samples were stored in microtubes containing lithium heparin (BD Microtainer; Becton Dickinson). The samples were placed in refrigeration a few minutes after being collected, and once all the samples had been taken for the day, they were transported in a thermal container with freezers to the Hema Veterinary Diagnostics SC clinical laboratory to perform their respective blood counts. The average time to transport the sample to the laboratory was 1 to 2 hours. The PCV was determined with a centrifuged capillary, and the MCV was calculated with the values of the Hct and the RBC count ($Hct \times 1,000 / Ery$). Total solids were determined by refractometry (refractometer for serum protein and urine specific gravity; TekcoPlus Ltd). Blood smears were made and evaluated with rapid Romanowsky hematology staining (RAL Diff-Quick; Siemens Healthineers). Total RBC and WBC counts were obtained using a manual method: the blood in the heparin tube was

Table 1—Comparative hematological values of blood samples collected from 21 lilac-crowned parrots (*Amazona finschi*) under physical (PR) and chemical restraint (CR) with isoflurane.

Analyte	PR (means ± SD)*	CR (means ± SD)*	PR (95% CI)	CR (95% CI)	P Value
PCV (L/L)	0.46 ± 0.04 ^a	0.44 ± 0.042 ^b	0.44-0.48	0.42-0.46	< .001
RBC (X 10 ¹² /L)	2.30 ± 0.30 ^a	2.12 ± 0.22 ^b	2.16-2.43	2.01-2.22	< .001
MCV (fL)	204.38 ± 21.38	209.38 ± 18.89	194.64-214.11	200.78-217.98	.87
WBC (X 10 ⁹ /L)	4.97 ± 3.33 ^a	4.2 ± 2.48 ^b	3.45-6.49	3.13-5.40	.01
Heterophiles (X 10 ⁹ /L)	3.73 ± 3.11	3.43 ± 2.21	2.31-5.14	2.42-4.44	.15
Lymphocytes (X 10 ⁹ /L)	0.96 ± 1.12 ^a	0.69 ± 0.70 ^b	0.45-1.47	0.37-1.01	.02
Monocytes (X 10 ⁹ /L)	0.07 ± 0.10	0.11 ± 0.16	0.03-0.12	0.04-0.19	.90
Eosinophils (X 10 ⁹ /L)	0.02 ± 0.04	0.02 ± 0.03	0.00-0.04	0.00-0.03	.38
Total solids (g/L)	45.42 ± 4.86 ^a	41.42 ± 4.84 ^b	43.21-47.64	39.22-43.63	< .001

*Means in the same row with different letters are significantly different ($P < .05$) between the sample collected under physical restraint and the sample collected under chemical restraint (30 minutes after induction).

mixed 1:200 with Natt-Herrick solution and then placed in a hematocytometer.

For the RBCs, the square in the center of the chamber was visualized, and RBCs were counted in 4 corner squares and the central square. This number was multiplied by 10,000 and the total number of RBCs per microliter was obtained. In the case of the WBCs, these were counted in the 9 largest fields of the camera, 10% was added, and the result was multiplied by 200 to obtain the total number of leukocytes per microliter.¹⁵ To determine the leukocyte differential, 100 cells were counted in the smear utilizing a microscope to establish the percentage and absolute values of each cellular component.

Statistical analysis

The blood count data collected under physical and chemical restraint were analyzed with the Shapiro-Wilk test to assess normality. The t test was used for related samples in the variables with proximate normal distribution, and the Wilcoxon test was used for those that did not exhibit a normal distribution. Statistical significance was established at $P < .05$. The information was analyzed using the statistical package JMP version 14.0 (SAS Institute Inc, 2018).

Results

CBCs were collected and analyzed from 21 lilac-crowned parrots (*A finschi*). The analytes that were significantly lower in samples collected under chemical restraint were PCV ($P < .001$), RBC ($P < .001$), WBC ($P = .01$), lymphocytes ($P = .02$), and total solids ($P < .001$). No significant differences were observed in MCV, heterophils, monocytes, and eosinophils (**Table 1**). Thrombocyte data were not evaluated because aggregates were formed in all samples and basophils were not found in any smear. Two of the birds died 7 days and 14 days after the procedure, respectively; their results were included in the study because they met the inclusion criteria, and the sample collection was performed according to the established methods.

The average anesthesia induction and recovery time was 3 minutes and 10 minutes, respectively. Some of the birds had apnea during the 30-minute maintenance time, so they were given assisted ventilation and lower isoflurane dosage.

Discussion

The results of this study show that physical restraint and inhaled anesthesia with isoflurane in lilac-crowned parrots (*A finschi*) affect some CBC analytes. Compared with the bird under physical restraint, the PCV, RBCs, WBCs, lymphocytes, and total solids were lower under chemical restraint. The diminished PCV value, RBC count, and total solids under chemical restraint with isoflurane agree with other similar studies performed on birds like dusky-legged guans (*P obscura*),¹¹ owls (*B virginianus*),¹⁰ falcons (*F sparverius*),¹² and eagles (*S cheela hoyo*)¹³ and mammals like guinea pigs (*C porcellus*),⁵ rats (*R norvegicus*),^{8,9} bats (*P hypomelanus* and *A jamaicensis*),^{6,7} goats (*Capra hircus*),¹⁶ sheep (*Ovis aries*),¹⁷ and humans (*Homo sapiens*).¹⁸ This could be associated with hemodilution caused by isoflurane, due to the decrease of blood pressure, increased vasodilation, and less peripheral vascular resistance which reduces the hydrostatic pressure causing fluid changes in the extravascular and intravascular spaces.¹⁸⁻²⁰ It is also suggested that this type of anesthetic causes splenic sequestration of RBCs in mammals,¹² but this reaction is unknown to occur in the spleen of birds. The study performed on owls including the American owl (*B virginianus*), eastern owl (*Megascops asio*), barred owl (*Strix varia*), barn owl (*Tyto alba*), and white owl (*Bubo scandiacus*) found that PCV decreased significantly in 60-minute prolonged anesthetic restraint with isoflurane and short 15-minute anesthetic restraints repeated for 3 days.

Likewise, the PCV was less in the 30-minute chemical restraint in this study on lilac-crowned parrots (*A finschi*). However, PCV alterations in the short 15-minute physical restraint protocols were not found in owls (*B virginianus*).¹⁰ These results suggest that longer anesthesia times with isoflurane may lower the PCV value. The falcon (*F sparverius*) study¹² proposed that PCV decreased in samples taken under physical restraint due to the amount of blood collected. This could affect the analyte measurement and cause iatrogenic anemia. This type of anemia is commonly reported in humans who have frequent or high-volume blood sample collections monitored under hospital supervision.^{21,22} Nevertheless, in birds, only 1% of the total weight

can be extracted to avoid a significant blood volume decrease.² In total, a maximum of 1 mL (0.3% of their weight) of blood was collected in lilac-crowned parrots in this study, whose weight was 300 g on average, so a dilution effect due to this cause is unlikely to happen.

As in our study, decreased WBC and lymphocyte counts were also found in owls (*B virginianus*),¹⁰ sheep (*O aries*),¹⁷ goats (*C hircus*),¹⁶ and bats (*P hypomelanus*).⁶ It is proposed that inhaled anesthesia causes this decrease due to its immunosuppressive effects.²³ In humans,²⁴ it is reported that in vitro both sevoflurane and isoflurane cause apoptosis of peripheral blood lymphocytes.

In a study in canines,²⁵ the immunosuppressive effects of propofol and isoflurane were compared; after 2 hours of anesthesia, isoflurane decreased the lymphocyte count in CBC more significantly than propofol, showing greater immunosuppression. In this case, such reduction was associated with the fact that the group studied with isoflurane had lower expression of proinflammatory cytokines (IL-2) and an increase of anti-inflammatory cytokines (IL-10). In owls¹⁰ (American owl [*B virginianus*], eastern owl [*M asio*], tawny owl [*Strix varia*], barn owl [*Tyto alba*], and snowy owl [*Bubo scandiacus*]), a decrease in heterophil measurements and fluctuating changes in eosinophils were observed in the protocol consisting of 15-minute chemical restraints for 3 consecutive days. This was related to the possible existence of an immunosuppressive response like that observed in neutrophils in mammals; however, changes caused by isoflurane specifically in heterophils and eosinophils need to be investigated to confirm this association.

Regarding manual restraint, a study in pigeons (*Columbia livia domestica*)²⁶ shows that handling these birds for 20 minutes in total, together with transportation for 3 hours increases heterophil and lowers lymphocyte counts compared with their baseline sample. Likewise, Amazon parrots (*Amazona ventralis*)²⁷ exposed to handling, transportation, physical exam, beak, and nail trimming, demonstrated an increase over time and a significant increase at the 20-minute sample in WBC and heterophil counts, while at the 40-minute sample lymphocyte percentage showed a significant decrease. In addition, plasma corticosterone also increases significantly in this Amazon species with at least 15 minutes of manual restraint.²⁸

These values were compared with their baseline sample taken under a 3-minute physical restraint in both studies,^{27,28} and the alterations were associated with a rise in stress levels. Although in the present study manual restraint was performed for 5 to 10 minutes, it is not ruled out that the results in WBCs, heterophils, and lymphocytes were not altered by stress (increased in the first 2 and decreased in the last). Possibly the use of isoflurane for 30 minutes masked the increase in heterophils and was not the main cause of the lymphocyte count decrease.

One of the limitations of this study was that CBCs were not performed at the time of blood collection but approximately 1 to 2 hours later. This caused

the thrombocytes to aggregate, making it difficult to count them; this effect is common when using lithium heparin as anticoagulant.²⁹ Consequently, for future studies, it is recommended to process the CBC as soon as the sample is collected, as well as to perform the smear without anticoagulant. Another limitation is that only 1 sample was collected during the anesthetic procedure at 30 minutes; therefore, it is suggested that in subsequent investigations blood samples be obtained every 5 to 10 minutes during anesthetic maintenance and after recovery. This will allow us to know the time at which the first significant changes in the analytes are noted and the time at which they return to baseline values. These findings obtained in *A finschi* suggest that there is variation in CBC values in blood samples collected under inhaled anesthesia with isoflurane in psittacines, and it could be possible that when the data are analyzed, they may not represent the accurate hematologic state of the patient. Consequently, it is important to know the method of restraint for blood collection when interpreting the CBC in this order of birds.

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