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Case Report

Asthma in an Adult Female Vervet Monkey (*Chlorocebus sabaeus*)

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A 9-y-old, colony-bred, female vervet monkey (*Chlorocebus sabaeus*) presented with a 6-y history of open-mouth breathing, tachypnea, and sibilant wheezing. These symptoms did not significantly affect her activity or quality of life. Thoracic radiographs and results of bronchoalveolar lavage supported the diagnosis of asthma. Treatment comprising intramuscular prednisolone (tapered over 2 mo from twice daily to every other day), inhaled salmeterol–fluticasone (25 µg–250 µg per actuation twice daily) by mask, and a metered dose inhaler was successful in restoring a normal respiratory pattern. Despite the availability of several primate models of human asthma, this case represents the first report of spontaneous asthma in a NHP.

Abbreviation: BAL, bronchoalveolar lavage.

Asthma is described as an inappropriate type 2 T-helper cell immunologic response to airborne allergens that manifests in chronic structural, inflammatory, and functional changes of the airways characterized by airway eosinophilia and hyperresponsiveness.[5] Asthma in NHP has only been described in research models. Allergen (pollen, house-dust mites, and *Ascaris suum*)-sensitized NHP, including squirrel monkeys (*Saimiri sciureus*), rhesus macaques (*Macaca mulatta*), and cynamolgus monkeys (*M. fascicularis*), have been used as animal models of asthma.[12] These NHP models have similar features to humans with asthma, in terms of early and late phases of bronchoconstriction (dual responders), response to allergen inhalation, and airway hyperreactivity. In addition, the inflammatory infiltrate of the airways is eosinophilic and neutrophilic in both NHP and humans.[10,19,25]

Case Report

A colony-born, multiparous, 9-y-old female vervet monkey (*Chlorocebus sabaeus*) housed in an outdoor breeding group at the Behavioural Science Foundation (St Kitts, West Indies) presented to the Ross University School of Veterinary Medicine hospital with a 6-y history of respiratory signs that did not affect her quality of life. The vervet monkey was housed and managed at the Behavioural Science Foundation in accordance with an IACUC-approved protocol, the Animal Welfare Act, and guidelines regarding the care and use of laboratory animals.[1,11] The clinical signs in this monkey had progressed gradually from intermittent paroxysmal episodes to continuous signs severe enough to warrant investigation. The respiratory problems were described as tachypnea, wheezing, open-mouth breathing and a nonproductive cough but no cyanosis. There was no noticeable exercise intolerance or change in behavior or appetite during the 6-y history of signs.

Cageside clinical examination confirmed that the vervet monkey was bright, alert, and responsive and had a body condition score of 2.5 (on a scale of 5) according to an NHP scoring system.[22] Examination of her respiratory pattern and auscultation detected expiratory effort with abnormal lung sounds, both audible and through auscultation, which were described as a sibilant wheeze starting at the end of inspiration and continuing through expiration.

Preliminary investigation, including blood sampling, radiography, and echocardiography, were performed under general anesthesia. The vervet monkey was placed in a squeeze cage for transport and administration of an immobilizing agent. After appropriate restraint within the cage, the subject received a combination of ketamine (7 mg/kg) and xylazine (0.6 mg/kg) into the quadriceps femoris muscle. She remained within the cage in a quiet, isolated environment for 10 min for development of sufficient immobilization. Immobilization was confirmed as a lack of response to both tactile and auditory stimulation. Oxygen supplementation was supplied at 3 L/min while the monkey was in lateral recumbency by using a pediatric rebreathing hose attached to a pediatric mask. Respiratory rate, heart rate, noninvasive blood pressure (oscillometric), and the echocardiogram were monitored and assessed every 5 min, and results were within the expected reference range. A 6-lead EKG was performed, and a mean heart rate of 153 bpm with a sinus rhythm was noted. Normothermia was maintained by using an air-free warming device (HotDog, Eden Prairie, MN) set at 104 °F (40 °C).

Laboratory tests included a CBC and biochemistry testing on blood collected from a peripheral vein collected under general anesthesia (Table 1). In addition to a stress leukogram, subclinical dehydration was diagnosed given the presence of mild relative

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Table 1. Laboratory test results

	Patient	Reference interval
Complete blood count		
RBC count	**6.34 × 10^{12}/L**	4.8–6.3 × 10^{12}/L
Hgb	**15.5 g/dL**	8.0–15 g/dL
Hct	43.43%	30%–44%
MCV	68 fL	50–90 fL
MCH	24.4 pg	18–36 pg
MCHC	35.7 g/dL	30–40 g/dL
RBC distribution width	16.1%	
WBC count	**16.18 × 10^{9}/L**	6–16 × 10^{9}/L
Mature neutrophils	**12.67 × 10^{9}/L**	1.9–6.2 × 10^{9}/L
Monocytes	**2.15 × 10^{9}/L**	0.04–0.72 × 10^{9}/L
Lymphocytes	**1.37 × 10^{9}/L**	4.3–9.2 × 10^{9}/L
Eosinophils	0	
Basophils	0	
Platelet count	382 × 10^{9}/L	200–600 × 10^{9}/L
Mean platelet volume	8.7 fL	
Platelet distribution width	34.7%	
Serum biochemistry		
Albumin	4.7 d/dL	2.9–4.7 g/dL
ALP	119 U/L	73–210 U/L
ALT	86 U/L	38–124 U/L
Amylase	363 U/L	149–500 U/L
Total bilirubin	0.4 mg/dL	0.1–0.4 mg/dL
BUN	**32 mg/dL**	12–30 mg/dL
Total calcium	**10.3 mg/dL**	7.7–9.4 mg/dL
Ionized calcium	**0.91 mmol/L**	1.00–1.5 mmol/L
Phosphorous	1.4 mg/dL	1.9–7 mg/dL
Creatinine	**0.3 mg/dL**	0.5–1.2 mg/dL
Glucose	80 mg/dL	50–100 mg/dL
Sodium	139 mmol/L	146–156 mmol/L
Potassium	4.3 mmol/L	2.9–6.5 mmol/L
Total protein	7.6 g/dL	6.1–7.5 g/dL
Globulin	2.8 g/dL	1.9–4.0 g/dL
Lingual blood gas (venous)		
pO_2	80 mm Hg	
pCO_2	**77 mm Hg**	
HCO_3	**31.8 mmol/L**	
pH	**7.22**	
Alveolar:arterial gap	**487**	
Fecal analysis		
Baermann fecal larval migration[a]	Negative	
Fecal flotation[b]	*Trichuris* spp. ova	

Abnormal results are highlighted in bold.
[a]3 consecutive samples
[b]Double centrifugation using Sheather sugar solution; 3 samples

polycythemia and increased BUN. The high total calcium concentration prompted measurement of the ionized calcium concentration, which was below the reference range; consequently the high total calcium value was assumed to be a spurious error. Baermann fecal larvae migration tests and fecal flotations using Sheather sugar solution and double centrifugation were performed on fresh stool collected under each of the 2 anesthetic episodes, with a final sample collected from the vervet monkey's enclosure, to screen for *Filariopsis* spp. and other lungworms; samples were collected 1-wk intervals (Table 1). There was no evidence of larvae, but *Trichuris* spp. ova, frequently diagnosed in this troop, were found.

Two orthogonal thoracic radiographs were obtained by direct digital radiography while the vervet monkey was immobilized (Figure 1 A and B). Radiologic findings included soft-tissue opacity around the pulmonary vessels, a mild bronchial pattern affecting the dorsocaudal lung fields, a flattened diaphragm, and a barrel-shaped thorax.

An echocardiogram (B-mode, M-mode and Doppler) was performed during the same anesthetic event and contractility appeared normal (Table 2). The reference ranges used were those for sedated cynomolgus monkeys (*Macaca fascicularis*).[21] There were no visible abnormalities (B-mode or Doppler) consistent with pulmonary hypertension that might explain the dyspnea.

A second procedure was conducted under general anesthesia 1 wk later to confirm the suspicion of asthma after other differential diagnoses had been excluded and to enable premedication with bronchodilators to mitigate the bronchoconstriction that is anticipated after bronchoalveolar lavage (BAL). After immobilization, in an identical manner to that described for the first procedure, the animal was nebulized with albuterol (0.5 mg in 4 mL saline) prior to bronchoscopy and BAL, by using a tight fitting face mask. A 22-gauge, 1-in. catheter was placed in the subject's left saphenous vein. A balanced crystalloid solution was administered at a rate of 5 mL/kg/h IV. The vervet monkey was then placed in a supine position, with her head and neck extended. Propofol was administered intravenously slowly to effect, to a total of 3 mg/kg. The larynx was visualized with the use of a Wisconsin-style laryngoscope blade with the anesthesiologist standing behind the subjects head. A dose of 0.2 mL 2% lidocaine was sprayed on the arytenoids to prevent the occurrence of laryngospasm. A sterile 3.5-mm, cuffed, Murphy-style endotracheal tube was placed within the trachea. During bronchoscopy and BAL, the patient was maintained on a light plane of anesthesia with the use of propofol intravenously titrated to affect. A transmission pulse oximetry probe was placed on patient's buccal mucosa to monitor oxygen saturation. After completion of BAL, the patient was connected to a pediatric rebreathing circuit with an inspired O_2 fraction of 100% and an expiratory sevoflurane concentration sufficient to maintain a light plane of anesthesia (that is, 2.0% to 2.5%). A venous blood gas sample was acquired from the lingual vein while the monkey received an inspired O_2 fraction of 93% during spontaneous ventilation. Respiratory acidosis was detected, given the venous pCO_2 and pH (Table 1).

A bronchoscope with an outer diameter of 5 mm was used to examine the airways but could not penetrate deeper than the mainstem bronchi because of the small size of the patient. The scope was not placed within the endotracheal tube due to size constraints and therefore was used without intubation at this time. The surfaces of the trachea and mainstem bronchi were

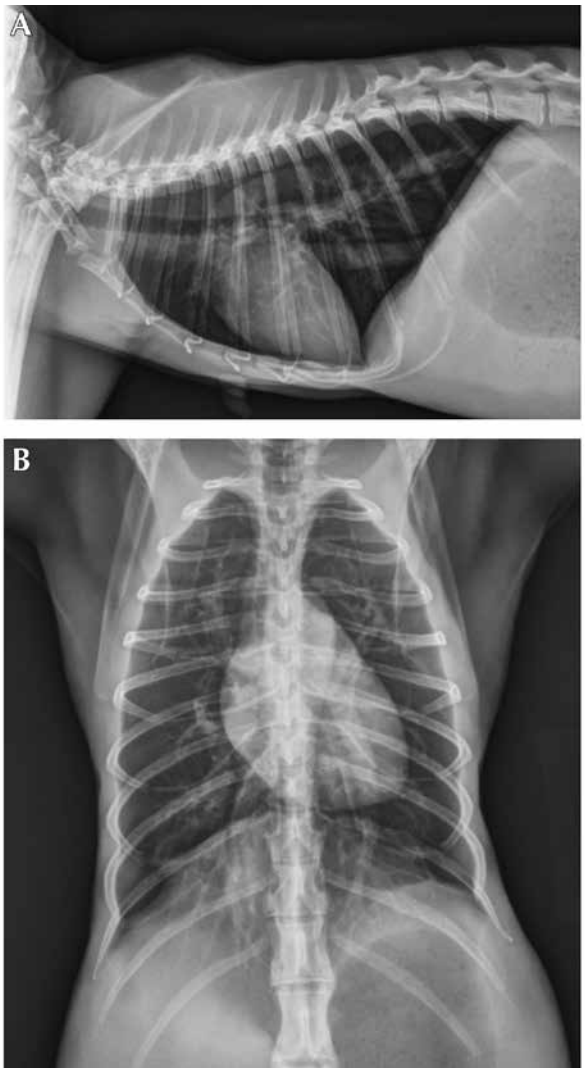

Figure 1. (A) Right lateral and (B) dorsoventral thoracic radiographs obtained while the monkey was sedated. Radiologic findings included soft-tissue opacity around the pulmonary vessels and a mild bronchial pattern affecting the dorsocaudal lung fields. The vertebral heart score was 9.9 (normal range, 9.0–10.40). The left cranial pulmonary artery was small (0.64 cm; normal range 0.80–1.40 cm). The diaphragm was flattened, contrary to the normal convex shape. On the dorsoventral view, the thorax had a barrel shape.

moist and glistening without visible mucus but bled easily after being touched gently with an instrument. Under endoscopic guidance, BAL was performed, with sampling the upper and intermediate bronchus and the upper and lower bronchus through the right and left lung mainstem bronchi, respectively, by using 5-mL aliquots of 0.9% saline solution, with a total of 20 mL instilled. A total of 8 mL of BAL fluid was retrieved and submitted for cytology and aerobic and fungal culture. The cytology sample was mildly cellular with a background of erythrocytes. The nucleated cells were predominantly eosinophils (69%), with 1 band eosinophil noted and a few ciliated columnar respiratory epithelial cells (4%) were present individually and in small sheets. Occasional small lymphocytes (12%) and reactive lymphocytes (3%) were noted, and neutrophils were rare. Macrophages (9%) occasionally contained hemosiderin pigment.

Table 2. Ultrasonographic findings

	Patient	Reference range
E-point septal separation	**1.9 mm**	0.0–1.3 mm
Interventricular septum diastole	**7.0 mm**	2.9–4.1 mm
Left ventricular diameter diastole	14.2 mm	12.0–15.6 mm
Left ventricular free wall diastole	**6.2 mm**	3.0–4.0 mm
Interventricular septum systole	6.2 mm	
Left ventricular diameter systole	8.0 mm	6.7–10.1 mm
Left ventricular wall systole	7.0 mm	
Fractional shortening	44%	32%–47%
LA/Ao (2D)	1.22	1.14–2.39
Ao (2D)	9.4 mm	
LA (2D)	11.5 mm	
Pulmonary outflow	0.75 m/s	
Tricuspid valve	0.59 m/s	
Aortic outflow	0.62 m/s	
Mitral valve	0.46 m/s	

B-mode, M-mode, and Doppler echocardiography of a ketamine–xylazine-immobilized vervet monkey (*Chlorocebus pygerythru*) with clinical signs of chronic respiratory distress. Reference ranges for cynomolgus monkeys were used as a reference, because reference intervals for vervet monkeys are unavailable. There was no indirect evidence of pulmonary artery hypertension, because no measurable tricuspid insufficiency with adequate contractility was noted. Abnormal results are highlighted in bold.

Aerobic culture did not yield growth after 72 h; *Paecilomyces* spp., a soil saprophyte, grew on Sabourad dextrose agar and was assumed to be a contaminant.

The vervet monkey was treated with prednisolone (0.5 mg/kg IM twice daily), inhaled corticosteroids (fluticasone, 250 µg/actuation, twice daily) by using a metered dose inhaler (Aerokat, Trudell Medical International, Ontario, Canada), and the bronchodilator salmeterol (25 µg/actuation). Achieving compliance with the inhaler was challenging, despite various methods of positive reinforcement; at the time of writing, management therapy was largely dependent on the intramuscular route. Prednisolone therapy achieved significant improvement of the breathing pattern and effort, with resolution of the sibilant wheezing and open-mouth breathing.

Discussion

To our knowledge, this case report is the first description of spontaneous asthma and the radiographic changes of asthma in an NHP. The radiographic changes in NHP used as models for asthma or spontaneously acquired asthma have not previously been documented.[15] The soft-tissue opacity around the vervet's pulmonary vessels and the mild bronchial pattern affecting the dorsocaudal lung fields might indicate mucous plugs within the airway or bronchial wall thickening. In addition, this NHP manifested the hallmark radiologic features of asthma in other species, including cats, that is, a flattened diaphragm and, in the dorsoventral view, a barrel-shaped thorax.[15]

The functionality of venous blood gases have been evaluated in emergent human subjects.[14] Venous pH, pCO_2, and bicarbonate values show a 95% agreement when compared with that those of arterial pH, pCO_2, and bicarbonate levels, respectively.[14] In addition, lingual venous blood gases are considered a clinically

acceptable substitute for arterial blood gas for measuring pH, pCO_2, and base excess but not pO_2.[16] The venous pO_2 value of 80 mm Hg with a fractional inspired oxygen concentration of 1.0 is lower than reported venous pO_2 values for humans under general anesthesia.[9,16] This relative hypoxemia might represent comorbid parenchymal or interstitial pathology; it might also be due to the method of sampling. The majority of human asthmatic patients present with hypocapnia and, less commonly, hypercapnia.[17,20] In the current NHP case, the patient was significantly hypercapnic. Increased pCO_2 has been documented in asthmatic human patients with a deteriorating clinical condition.[18] Asthmatic hypercapnia may be a result of decreased stimulation of the respiratory muscles from the CNS to avoid terminal muscle fatigue and preserve muscle function.[26] Alternatively, the increased pCO_2 in this vervet monkey might reflect the use of anesthesia (but likely not to this severity) or airway reactivity; status asthmaticus in humans can manifest as respiratory acidosis.[2]

Because sevoflurane inhalation and ketamine can ameliorate the clinical signs and decreased pCO_2 in children with status asthmaticus, the pCO_2 in this monkey when conscious might be even more severe without the benefit of inhaled sevoflurane.[7,20] Another consideration regarding the anesthetic choice is its effect on echocardiography. Although pulmonary hypertension was excluded on echocardiography, the references ranges used do not take into account xylazine administration in NHP. However, the effects of ketamine and xylazine on pulmonary artery hypertension in pigs were shown to be nonsignificant when compared with baseline values.[23]

The *Paecilomyces* spp. isolated during BAL of our vervet monkey were presumed to be a contaminant. *Paecilomyces* spp. can cause infection in immunocompromised humans, including those with organ transplants, hematologic malignancies, diabetes mellitus or chronic steroid use; none of these conditions was recorded in this vervet monkey.[24] If ubiquitous in the animal's environment, this organism might be the primary trigger for her allergic airway disease.

A diagnosis of asthma was made in light of the animal's wheezing, expiratory effort, radiologic signs, eosinophilia on BAL cytology, and response to corticosteroid treatment. Potential differentials for this condition include eosinophilic bronchitis, which has previously been reported as a cause of nonresponsive respiratory distress in a colony-born *Macaca mulatta*, and *Filariopsis* spp. lungworm infestation, which has occurred in white-faced capuchin monkeys (*Cebus capucinus*).[4,13] Eosinophilic bronchitis in humans is a benign condition that is caused by occupational hazards that is characterized by a lack of bronchial hyperresponsiveness—and thus unlikely in the present case—although the condition in some patients has progressed to chronic obstructive pulmonary disease, which is characterized by a corticosteroid-responsive chronic cough.[4] A similar condition in dogs, eosinophilic bronchopneumopathy, is characterized by an overwhelming eosinophilic inflammation on BAL cytology and an alveolar, interstitial, or bronchial lung pattern but lacks airway reactivity.[6] Although not performed in our case, pulmonary function testing would be definitive, and therefore airway hyperresponsiveness could not be confirmed, but the audible sibilant wheezing was indicative and the resolution of this clinical sign after bronchodilator therapy supports this type of pathology.

As in humans with atopic asthma, NHP with experimentally induced asthma, the patient's temperamental pattern, specifically reduced emotionality and increased vigilance, is associated with autonomic reactivity and airway responses during stressful situations.[3] However, the monkey in our case report is not a particularly withdrawn animal, and her temperament was unlikely to have contributed to the manifestation of her disease.

Treatment for asthma in humans comprises inhaled corticosteroids, β_2-adrenergic receptor agonists, and oral leukotriene-receptor blockers.[8] A similar drug protocol was used in the current case, with an excellent and rapid response. The challenging aspect of managing this patient was the low tolerance for the face mask and metered dose inhaler despite positive reinforcement training. A limitation of this case study was the lack of a lung function study by spirometry; although noninvasive, spirometry likely would be challenging in NHP due to their lack of cooperation. An alternative is a forced oscillation technique, which measures impedance, from which airway resistance could be calculated.[5]

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