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Case Report

Ventricular Parasystole in a Neonatal Rhesus Macaque (*Macaca mulatta*)

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A 6-d-old Indian-origin female rhesus macaque (*Macaca mulatta*) presented with bradycardia shortly after sedation with ketamine. No other cardiac abnormalities were apparent. Approximately 2 wk after the initial presentation, the macaque was again bradycardic and exhibited a regularly irregular arrhythmia on a prestudy examination. ECG, echocardiography, blood pressure measurement, SpO2 assessment, and a CBC analysis were performed. The echocardiogram and bloodwork were normal, but the infant was hypotensive at the time of echocardiogram. The ECG revealed ventricular parasystole. Ventricular parasystole is considered a benign arrhythmia caused by an ectopic pacemaker that is insulated from impulses from the sinus node. Given this abnormality, the macaque was transferred to a short-term study protocol, according to veterinary recommendation. On the final veterinary exam, a grade 3 systolic murmur and a decrease in arrhythmia frequency were noted. Gross cardiac lesions were not identified at necropsy the following day. Cardiac tissue sections were essentially normal on microscopic examination. This infant did not display signs of cardiovascular insufficiency, and a review of the medical record indicated normal growth, feed intake and activity levels. This case demonstrates the importance of appropriate screening of potential neonatal and juvenile research candidates for occult cardiovascular abnormalities. Whether the arrhythmia diagnosed in this case was truly innocuous is unclear, given the documented hypotension and the development of a systolic heart murmur.

NHP are important research models for heart disease, one of the leading causes of death in human adults. Macaque models of the progression and regression of diet-induced atherosclerosis are key to understanding the pathogenesis behind coronary artery disease and the development of potential treatment options.[22] Currently, cell-based therapies to induce cardiomyocyte regeneration are being studied in NHP models. However, the development of post transplantation arrhythmias is one of the main limiting steps in the translation of this treatment to humans.[3] This condition is a perfect example of how the development or clinical progression of an arrhythmia is difficult to predict even though the cellular mechanisms of arrhythmogenesis are relatively well understood.

It is important to differentiate background ECG abnormalities from research-related effects. Although ECG reference ranges have been established for several NHP species, age-specific normal values for the ECG parameters of neonatal NHP populations are sparse.[6,8,12,28] Much of what is known regarding common background ECG abnormalities in macaques was gathered from control animals in cardiotoxic drug assessment.[8] In a retrospective study evaluating arrhythmias in experimentally naive telemetered macaques, sinus arrhythmias such as wandering pacemaker, tachycardia, bradycardia, and sinus blocks were the most prevalent.[9] However, abnormal complexes including atrial and ventricular premature contractions and more clinically concerning arrhythmias, such as ventricular tachycardia and ventricular escape rhythms, were documented also.[9] These studies could have sweeping effects as a basis for new model development, given the US Food and Drug Administration's request for the development of alternative in vivo assays to the QT prolongation assay for assessing the proarrrhythmic nature of drugs.[7]

Expanding on this literature, the current report describes the clinical presentation, diagnostic findings, and associated pathology related to a rare arrhythmia, ventricular parasystole, in a neonatal rhesus macaque. Transient, innocuous neonatal arrhythmias are common in many species, including humans (up to 90%)[22] and foals (up to 95%).[28] However, persistent neonatal arrhythmias may indicate an underlying congenital abnormality or disease state. Ventricular parasystole is classically considered a benign arrhythmia that arises from the presence of an ectopic pacemaker site within the ventricles.[4] This ectopic pacemaker site operates independently of the baseline sinus rhythm and is considered to be insulated from alterations from surrounding electrical activity or autonomic innervation.[4] Although ventricular parasystole has been described in other species (humans,[2,18,23] horses,[24] cats, and dogs[5]), the current report represents the first published case in an NHP.

Case Report

A 6-d-old, Indian-origin female rhesus macaque was noted to be bradycardic (160 bpm) on the initial intake exam for removal from the dam and placement within the nursery. Nursery infants are housed in individual incubators and fed a commercial formula (Similac with Iron, Abbot Laboratories, Chicago, IL) prepared according to the manufacturer's recommendations every 2 to 4 h.

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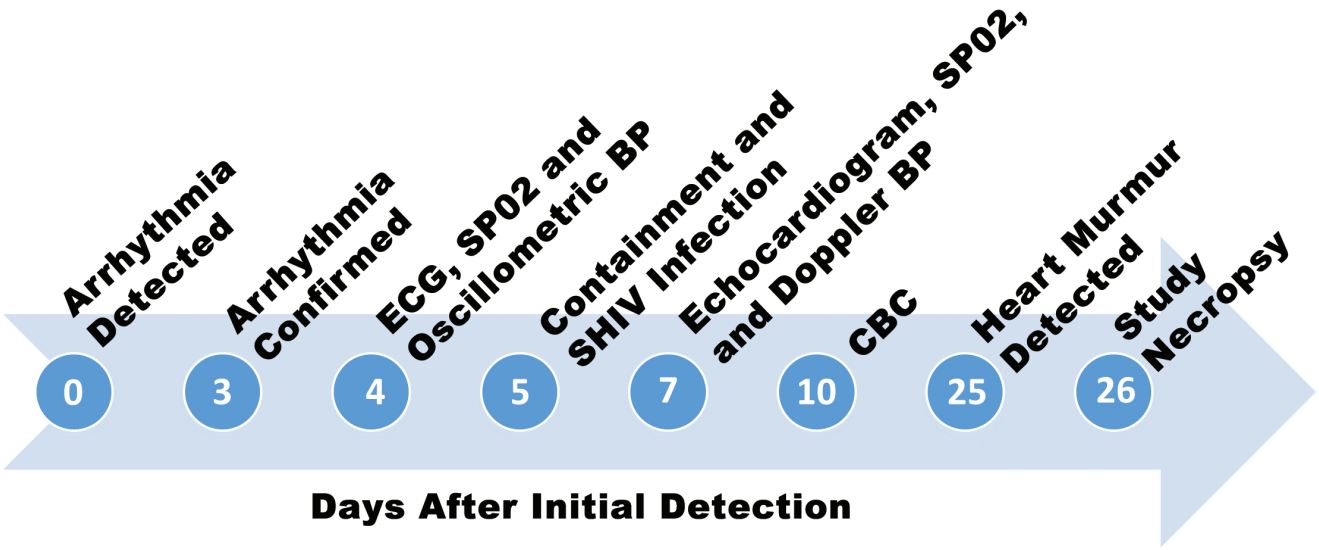

Figure 1. Timeline of diagnostic and research-related events from initial detection of the arrhythmia.

Food intake is noted at each feeding, and the infants are weighed every 2 to 3 d. This macaque was from an SPF outdoor colony monitored for SIV, simian T-lymphotropic virus, simian retrovirus, and *Macacine herpesvirus 1* at an AAALAC-accredited facility (Oregon National Primate Research Center, Beaverton, OR), and animals are maintained in accordance with the Animal Welfare Act and the *Guide for the Care and Use of Laboratory Animals*.[13,26] The protocol for the experimental manipulation of this animal was IACUC-approved.

At initial presentation, the macaque was experimentally naïve and had been sedated (10mg/kg IM ketamine; Zoetis, Florham Park, NJ) the same day for tattooing. In addition to the bradycardia (normal heart rates for neonatal rhesus macaques range from 200 to 240 bpm depending on age and time of day),[20] the only other findings on physical examination were mild dermatitis over the tail base and minimal umbilical swelling and irritation. The bradycardia initially was attributed to sedation; the infant handled the sedation well and had a normal recovery. Repeat auscultations were scheduled to determine if the bradycardia persisted. During 2 additional recheck auscultations, the heart rate was within normal limits (approximately 200 bpm), and the dermatitis and umbilical irritation had decreased. However, on the final prestudy exam less than 2 wk after initial presentation, the macaque was again bradycardic (140 bpm) and had developed a regularly irregular arrhythmia, which repeated 3 to 5 times each minute.

Given the clinical findings and potential for a congenital cardiac abnormality, the researcher was recommended to reassign the macaque to a short-term, terminal study. The animal was immediately enrolled in a month-long SIV–HIV vaccine trial and moved from the nursery to biocontainment prior to infection. Serial recheck examinations were performed without sedation, and diagnostic procedures included ECG, echocardiography, blood pressure measurement, indirect assessment of SpO_2, and CBC analysis to characterize the arrhythmia (Figure 1). ECG, pulse oximetry, and oscillometric blood pressure were acquired by using a portable monitoring device (SurgiVet Advisor Vital Signs Monitor, Smiths Medical, Dublin, OH). Parasternal long-axis and heart base short-axis standard echocardiographic views were obtained by using a portable ultrasound unit (GE Logiq E-Vet, Sound, Carlsbad, CA). An automated hematology analyzer (Pentra 60 C+, Horiba Medical, Irvine, CA) was used to perform the CBC with differential analysis.

The macaque's blood pressure and heart rate varied on subsequent exams and between modalities. Oscillometric blood pressure (105/60 and 99/45 mm Hg) indicated that the macaque was normotensive at the time of ECG. Although the heart rate was normal at this exam (180 to 200 bpm), the regularly irregular arrhythmia previously observed during bradycardic episodes was recorded on the ECG strip (Figure 2). Systolic blood pressure taken by Doppler sonography (Parks Medical Electronics, Aloha, OR) at the time of the echocardiogram was 40 to 50 mm Hg, indicating hypotension. However, the echocardiogram obtained at this time was inconclusive due to the limitations of the portable ultrasound unit including decreased image resolution from a low-Hertz probe and insufficient Doppler acquisition rate. The lack of concurrent ECG and M-mode readings during echocardiography further complicated interpretation. However, no marked structural or functional abnormalities were noted (Figure 3). Time and research constraints precluded repeat assessment of the ECG or echocardiogram. Consultation with a veterinary cardiologist regarding the electrocardiogram findings revealed that the macaque was affected with ventricular parasystole characterized by an interectopic interval of 520 centiseconds and a constant coupling interval of 300 centiseconds.

Additional diagnostics did not reveal an underlying systemic cause for this arrhythmia. CBC analysis demonstrated minimal to mild abnormalities, including lymphocytosis and increased Hct characterized by increased MCV and MCH (Table 1).

Throughout the study, the neonatal macaque continued to have a normal growth rate, as assessed by serial body weights comparable with others in the cohort, and never displayed signs of cardiovascular insufficiency, such as tachypnea, syncope, lethargy, and ascites. Given the lack of clinical signs and concerns for confounding research variables, no treatments for the arrhythmia were attempted. On the final veterinary examination, a grade 3 systolic murmur was present, and the arrhythmia frequency had diminished to less than 3 or 4 times per minute.

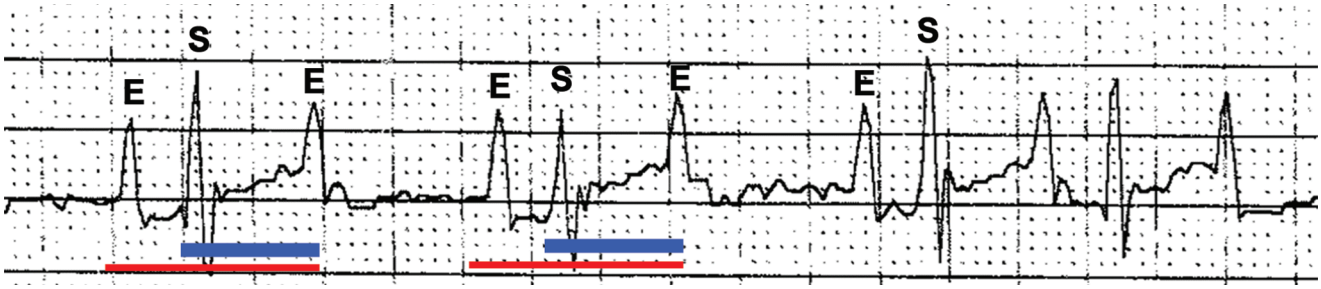

Figure 2. ECG of affected macaque displaying ventricular parasystole. S, Normal sinus beat; E, ectopic beat; red line, interectopic interval; blue line, coupling interval. Paper speed, 25 mm/s.

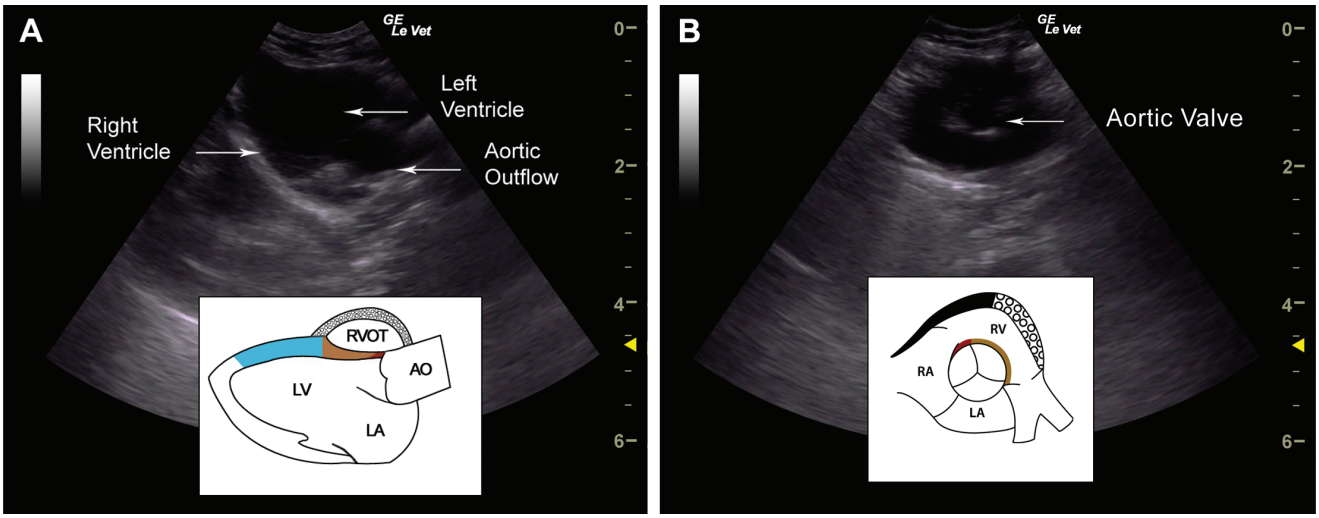

Figure 3. Echocardiographic images of the (A) parasternal long-axis and (B) heart base short-axis views. Representative images are included to highlight structures.[11] The parasternal long-axis image is slightly rotated compared with the representative schematic. None of the images revealed any obvious structural defects.

Table 1. CBC analysis of infant rhesus macaque with ventricular parasystole

	Value	Reference range	Assessment
Leukogram			
WBC (× 10^3/μL)	8.9	4.2–13.6	Normal
Neutrophils (× 10^3/μL)	3.55	2.38–9.61	Normal
Lymphocytes (× 10^3/μL)	4.8	1.13–3.77	Mild lymphocytosis
Monocytes (× 10^3/μL)	0.37	0.19–0.92	Normal
Eosinophils (× 10^3/μL)	0.06	0.04–0.83	Normal
Basophils (× 10^3/μL)	0.07	0.02–0.10	Normal
Hemogram			
RBC (× 10^6/μL)	5.41	4.5–5.84	Normal
Hct (%)	43.50	32.9–41.8	High
Hgb (g/dL)	13.8	10.7–13.8	Normal
MCV (fL)	85	65.6–76.4	High
MCH (pg)	26.9	21.2–25.2	High
Thrombogram			
Platelet number (× 10^3/μL)	389	119–471	Normal

Reference values were acquired from inhouse analysis of 107 adult female rhesus macaques ranging in age from 5 to 11 y; age-specific normal values are unavailable.

A complete necropsy was performed at the experimental endpoint. No gross cardiac abnormalities were noted. Representative tissues were collected, fixed by immersion in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin. Tissues from all 4 chambers including components of the cardiac conduction system (Figure 4 A)

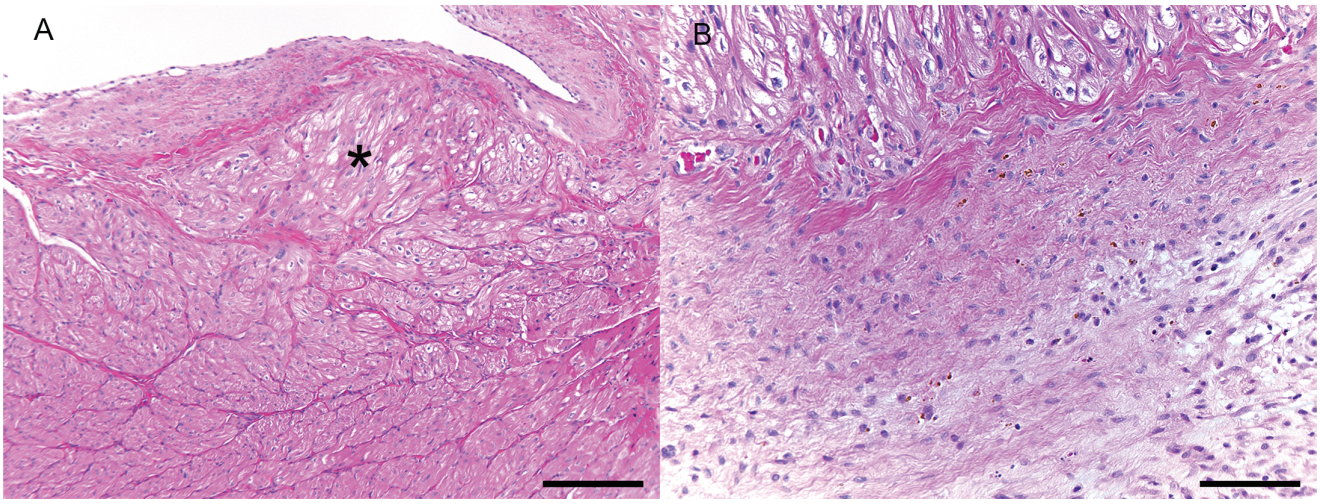

Figure 4. (A) Histology of normal, subendocardial, vacuolated specialized muscle fibers of the conduction system (*) and subjacent myocardium. Bar, 200 µm. (B) This section of right atrium contains fibroelastic subendocardial tissue, few hemosiderin-laden macrophages, and apoptotic cells. Bar, 100 µm. Hematoxylin and eosin stain.

were examined microscopically. Focally within the subendocardial tissue of the right atrium, there were minimal numbers of hemosiderin-laden macrophages and low numbers of apoptotic cells (Figure 4 B). In addition, other histologic findings of no clinical significance in this case included minimal to mild typhlocolitis and renal tubule degeneration, minimal renal interstitial fibrosis, mild thymic lymphoid depletion and minimal lymphoid hyperplasia of the splenic white pulp, gut-associated lymphoid tissue, and mesenteric lymph nodes.

Discussion

Ventricular parasystole is classically described as having 3 ECG features: consistent interectopic intervals, varying coupling intervals, and the presence of fusion beats.[15-17] The interectopic interval refers to the period between ectopic discharges. The consistent interectopic intervals arise from the presence of an entrance block, which insulates the ectopic pacemaker from alterations of its rate by discharges from the sinus rate.[27] This parameter is in contrast to the coupling interval, which refers to the time between the ectopic beat and the preceding sinus beat. Discharges from the ectopic pacemaker can send surrounding pacemaker cells into a refractory period, altering the underlying sinus rate. Fusion beats describe synchronous discharges by both the normal conduction system pacemaker and the ectopic foci, resulting in abnormal and increased-amplitude QRS complexes. Although cases of ventricular parasystole may display all 3 of these features, the macaque we report displayed only consistent interectopic intervals, the only feature required for diagnosis of ventricular parasystole, without the other ECG abnormalities. This unique presentation of ventricular parasystole has previously been reported only in humans.[10] Therefore the case we present here is the only reported veterinary case of ventricular parasystole with a fixed coupling interval.

Complex presentations of ventricular parasystole, such as the one in this case, are difficult to identify, given its low general prevalence even in the human population (0.13%).[4] In addition, diagnosis requires precise calculation of the interectopic interval, which can be confounded by concurrent arrhythmias or the presence of an exit block at the ectopic focus that would result in skipped ectopic beats.[27] Given these challenges, consultation with a veterinary cardiologist is recommended for any potential cases. However, when a potential case emerges, induction of a vagal response by using either a parasympathomimetic agent or by manual stimulation of the mammalian dive response can differentiate ventricular parasystole from ventricular premature contractions, which are much more common.[5,23] This response causes a brief sinus arrest which affects systolic beats like ventricular premature contractions. However, parasystolic beats will remain unchanged under vagal stimulation.[17] Because we were unable to repeat the ECG in this macaque, the vagal response test could not be performed.

We were unable to identify any systemic or pharmacologic causes of the arrhythmia in this macaque. The abnormal findings on the CBC were attributed to effects of age, research manipulations, or sample lysis on collection. Human newborns typically demonstrate a relative increase in Hct and MCV prior to normalization of these parameters and the emergence of lymphocytes as the dominant WBC type throughout most of childhood.[19] In addition, because this macaque was infected with a lymphotropic virus as part of its study, dynamic alterations in that cell population would be expected. Additional diagnostics would have been helpful to further characterize the arrhythmia, assess its effect on cardiac function, and identify possible underlying etiologies. However, because of interference with research aims or lack of feasibility, they were not pursued. The effects of additional blood collections on research aims precluded serum chemistry or cardiac biomarker assessment. In addition, cardiac biomarkers are limited as a clinical tool in NHP given a lack of correlation to nonacute myocardial injury and the inability to use human automated assays for some parameters.[25] Direct measurement of cardiac function, which requires surgical-plane anesthesia, was deemed unnecessarily invasive, and noninvasive Holter monitoring has not been optimized for neonatal macaques.

Grossly and microscopically the heart of this animal appeared essentially normal. The low numbers of hemosiderin-laden macrophages reflecting prior hemorrhage and the apoptotic cells present in the subendocardial atrial tissue on microscopic examination (Figure 4 B) do not account for the cardiac abnormalities diagnosed clinically. In humans, ventricular parasystole has been

documented in association with dilated cardiomyopathy and myocardial infarction.[14,21] Often there are no gross or histopathologic cardiac findings in cardiac deaths secondary to electrical conduction abnormalities, especially in human cases of sudden death in infants or young athletes.[23] Ventricular parasystole has been described as a benign, transient arrhythmia in neonatal human patients.[2] This condition has also been observed and reported as a benign, transient arrhythmia in association with equine colic.[25] Although there are anecdotal associations between ventricular parasystole and anesthesia in cats, we do not believe that anesthesia underlay the condition in this macaque. Even without clear pathologic evidence for cardiac or systemic disease, the macaque's clinically noted intermittent bradycardia, persistent arrhythmia, intermittent hypotension, and murmur development suggest that the arrhythmia was not benign. Had the macaque survived into maturity, pathologic sequelae might have resulted in overt cardiovascular insufficiency.

Although no treatment attempts were made in this macaque, ventricular parasystole typically is considered refractory to medical management with antiarrhythmics and β-blockers.[5] When required, surgical ablation to inactivate the ectopic pacemaker site is pursued.[14] Growing evidence in the human literature suggests a potential association between ventricular parasystole and more serious cardiac conditions, including acute myocardial infarction and other forms of heart disease.[1,21,23] Therefore, any case of ventricular parasystole requires a complete cardiac work up and subsequent follow-up monitoring.

In summary, this report describes the first case of ventricular parasystole in a NHP species and the only reported veterinary case of ventricular parasystole with a fixed coupling interval. In terms of a broader scope, this case exemplifies the need for serial cardiovascular screenings for neonatal and juvenile animals to identify occult cardiovascular abnormalities, such as ventricular parasystole, that would make them inappropriate research candidates. Finally, increased awareness of ventricular parasystole in veterinary species will aid in the interpretation of research-acquired ECGs, such as those performed during toxicologic drug testing or in cardiovascular research models.

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