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Possible asynchronous parturition in a multifetal strepsirrhine: *Hapalemur meridionalis*

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Abstract

Although infrequent among primates, twinning is common among strepsirrhines, a group of primates that includes the lemurs of Madagascar. As with any multifetal pregnancy, complications during gestation or even parturition can arise due to various factors (e.g., amniotic infection, stress). On rare occasions during labour of twins, parturition may be delayed between the two neonates, a phenomenon known as 'delayed interval delivery' that has been well-documented among the human medical journals. Based on circumstantial evidence, we report an opportunistic account of a habituated, adult female southern bamboo lemur (*Hapalemur meridionalis*) from Mandena, southeast Madagascar, first giving birth to an infant in mid-October 2013 (found deceased), and then birthing a live infant sometime between 5-21 days later. Similar to cases reported in human medical literature, the second infant survived and appeared to stay healthy until the conclusion of the study. Our account of asynchronous parturition of twins in a non-human primate is, to our knowledge, the first reported case in the literature.

Keywords: delayed interval delivery, pregnancy, southern bamboo lemur, twinning.

Introduction

The asynchronous delivery of human twins was first described as a coincidental event by Carson (1880) with 44 days occurring between births. Since then, the occurrence of this phenomenon (i.e., contractions cease between multifetal births) has been well documented within the human medical literature (reviewed in Wittmann *et al*., 1992) where it is more commonly referred to as 'delayed interval delivery.' The majority of this literature is composed of reports and small case studies that describe proactive management policies of intentionally delaying delivery after preterm parturition of a first twin; however, not all delayed interval deliveries are medically-inhibited by tocolytics, i.e., a labour suppressant medication (Wittmann *et al*., 1992). Whether natural or medically-inhibited, the cessation of labour after the delivery of a premature first twin has been demonstrated to show an improved fetal outcome for the second twin (Song *et al*., 2000; Wouters *et al*., 2009). According to a human population-based study, asynchronous parturition occurred in approximately 0.09% of multifetal pregnancies; however, it was not disclosed how many of these delays were due to human medical intervention (Zhang *et al*., 2004). To our knowledge the asynchronous birthing of twins has never been described in any non-human primates.

For this phenomenon to occur, the twins must be dizygotic. In short, dizygotic twins develop from two separate ova in the uterus, with each zygote developing its own amnion (i.e., amniotic sac) and chorion as part of two separate placentas. Placentas do not necessarily need to be un-fused for this phenomenon to occur, as among humans a fused placenta from the first twin can remain in-utero until the birth of the second (de Jong *et al*., 1995). Dizygotic twinning is much more common than monozygotic twinning in humans (Hoekstra *et al*., 2008) and is known to occur in other non-human primates (Geissmann, 1990; Ely *et al*., 2006; Harris *et al*., 2014). Twinning rates among haplorrhine primates are low (e.g., Geissmann, 1990; Link *et al*., 2006; Huck *et al*., 2014; but see Bales *et al*., 2001 for an example of callitrichid twinning), however, it appears to be extremely common in primates with a bicornuate uterus, such as in Strepsirrhini, i.e., a suborder of Primates which includes lemurs, lorises, and galagos (Pasztor and van Horn, 1976; van Horn and Eaton, 1979; Benirschke and Miller, 1982; Parga and Lessnau, 2005; Tecot *et al*., 2013).

In this report we present the first observation of a multifetal non-human primate delivering one offspring, and then giving birth to a second infant multiple days later.

Materials and Methods

We conducted observations on four social groups of southern bamboo lemurs (*Hapalemur meridionalis*) from October 2012 through December 2013 at the Mandena littoral forest (24°95'S 46°99'E) in southeast Madagascar. This protected area is located approximately 10 km north of Fort-Dauphin and is 230 ha of degraded littoral forest fragments and interspersed swamp (Eppley *et al*., 2015a). These relatively small-bodied primates (mean ± SD weight across sexes from wild: 1.072 ± 0.107 kg; n = 15; Eppley *et al*., 2015c) are characterized by folivory and exhibit a cathemeral

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activity pattern (Eppley *et al*., 2011, 2015b). They live in small social groups with one or two breeding females and typically one breeding male, with an average group size of 5.6 ± 1.5 individuals (n = 5; Eppley *et al*., 2016).

As part of a larger behavioural ecology research project, ten adult *H. meridionalis*, from across four neighbouring social groups, were captured and collared by an experienced Malagasy technician using a blowpipe and lightweight syringes (Telinject, USA) with a hypnotic anaesthesia 4 mg/kg of ketamine hydrochloride) between October and December 2012. Individuals were fitted with an external radio-transmitter (ARC400, Advanced Telemetry Systems, Isanti, MN, USA) with individual identification tags to assist in the initial habituation and in decreasing the amount of time it takes to locate the focal group each morning. Details of the capture protocol followed have been previously described in Eppley *et al*. (2015b), and collars were removed at the end of the study in December 2013 following the same protocol.

Focal data collection was limited to three *H. meridionalis* groups that resulted in approximately 50 h/month of data from full-day focal follows, providing an approximate total of 150 h/month. For the sake of this report, we will only describe the events that occurred while following one of these groups (M1) as collected *ad libitum*, between October and November 2013. *Hapalemur* live in stable social units (Tan, 2006); at the time of these observations, group M1 comprised one adult female (M1F2), one adult male (M1M2), and one juvenile female (M1F4) who was ≤1 year old.

This research was carried out under the Accord de Collaboration between the University of Antananarivo and the University of Hamburg. Research protocols were approved and permits authorized by Commission Tripartite of the Direction des Eaux et Forêts de Madagascar (Autorisation de recherché N° 240/12/MEF/SG/DGF/DCB.SAP/SCB du 17/09/2012), adhering to the legal requirements of Madagascar.

Results

We conducted a full-day follow (sunrise to sunset: 05:30 - 18:15) of the *H. meridionalis* group M1 on October 17, 2013 and all three individuals were present. Upon locating the M1 group in the center of their territorial home range at 08:00 on October 18, 2013, we first found the adult female (M1F2) sitting on a tree branch approximately three and a half meters high and looking at the ground. We discovered a neonate positioned ventrally on the forest floor nearby. The male infant, 42 g, was without a heart rate and it appeared rigor mortis had set in as the limbs remained rigid when handling the corpse. The body was for the most part dry and we were unable to locate the placenta. The two other individuals from the group were not in the immediate vicinity but were located in a sleeping huddle 30 m away. The dead neonate did not show any signs of wounding or aggression, but did exhibit a lack of fur on its ventral side and near hairless tail, in addition to peculiar small red bumps on the chest. Over the next four consecutive days, we conducted full-day follows with this group and can conclusively state that there were only three individuals within the M1 group during this time. On November 20th, we resumed our focal data collection with M1 group and found the adult female M1F2 now with an infant. This leads us to conclude that the sole adult female in the group was multifetal and that at some point between October 23rd and November 20th delivered a second, full-term neonate. At the conclusion of the study in early January 2014, the second infant was still alive and appeared in good health.

Discussion

To our knowledge, this is likely the first reported case of a natural delayed interval (asynchronous) parturition to occur in a non-human primate. It has been reported that twinning is common among *H. alaotrensis*, a close congener of *H. meridionalis* (Steyn and Feistner, 1994; Beattie and Feistner, 1998; Mutschler *et al*., 2000), and while it is less frequent among *H. griseus* (Haring and Davis, 1998), twinning is known to occur (Tan, 2000). Although lemurs are known for their high-frequency of multifetal pregnancies similar to some callitrichid primates (Bales *et al*., 2001; Harris *et al*., 2014), these also result in high infant mortality (Debyser, 1995; Parga and Lessnau, 2005; Volampeno *et al*., 2011). In some cases an accidental fall may cause or lead to death in a neonate (Morland, 1990), yet stillborn and infant mortality have been shown to increase with multifetal births, often with stress and infections linked to infant/juvenile mortality (Debyser, 1995). In our observation, the first neonate lacked ventral fur and had a near hairless tail, suggesting that it was delivered prematurely (Cummings *et al*., 2012). In any case, the occurrence of a delayed interval birth is exceedingly rare.

Most lemurs are sexually quiescent throughout much of the year and exhibit a strict seasonal breeding once per year, an adaptation resulting in mono-estrus and a short estrus period (Jolly, 1967; Rasmussen, 1985; Brockman and van Schaik, 2005). With an interbirth interval of one year, the same is true of *Hapalemur* spp. (Tan, 2000, 2006); however, the degree of seasonal stringency may be affected by resource abundance (Mutschler *et al*., 2000; Brockman and van Schaik, 2005). Similar to reports that *H. griseus* mates in June-July and gives birth in October-November (Tan, 2000), all adult female *H. meridionalis* within these neighbouring groups were first observed with infants from mid-November to mid-December (Table 1), making it likely that the dead neonate was in fact premature.

Table 1. Date of first observations of *H. meridionalis* infants in Mandena and their presumed mothers in 2013.

Date (of first sight)	Group	Mother ID	Outcome
October 18	1	M1F2	Died – likely premature stillbirth
November 15	2	M2F1	Alive through January 2014
November 19	4	M4F1	Died – unknown cause
November 20	1	M1F2	Alive through January 2014
December 14	4	M4F2	Alive through January 2014

Note: These were the only adult *H. meridionalis* females in our focal groups.

While we cannot conclusively state that both infants were birthed by M1F2 due to lack of genetic samples, strong evidence supports our assertion. First, the adult female M1F2 was noticeably larger preceding the discovery of the dead neonate in October and infant in November. Second, *Hapalemur* spp. are highly territorial and protect their territory via agonistic encounters with neighbouring bamboo lemur groups (Nievergelt *et al*., 1998; Tan, 2006). Similar to congeners, *H. meridionalis* in Mandena maintain minimal home range overlap between groups (Eppley *et al*., 2015a), leading groups to rarely come within visual distance of others. Due to this, *H. meridionalis* exhibit extremely low rates of inter-group agonism in Mandena (Eppley *et al*., 2016). Furthermore, the site of the dead neonate was in the central core area of group M1's territory, a relatively large distance (approximately 150 m) from the shared borders with neighbouring groups. Thus, it seems unlikely that an out-group adult female would travel to the center of another group's territory to give birth. Third, though infanticide has been observed among some lemur species (reviewed in Tecot *et al*., 2013), the dead neonate showed no signs of wounding or aggression, therefore making it unlikely that the infant was killed by its own or a neighbouring group.

Our report being an observation among wild primates leaves us in a difficult position to speculate the circumstances surrounding how and why this occurred. It is possible that the cost of enduring a multifetal pregnancy could be too great if certain resources are not as abundant. Numerous health issues can arise from a multifetal gestation, placing morbidity or mortality risks on the fetuses and mother (Kalchbrenner *et al*., 1998). It is possible that preterm infection (potentially indicated by the observed red bumps across the neonate ventrum) could have been responsible for the infant's death (Darmstadt *et al*., 2000), whether stillborn or shortly after birth. The offspring of primate mothers who experienced prenatal stress may have delayed motor activity or other long-term morbidities as a result (Schneider *et al*., 1999; Mulder *et al*., 2002). Premature birth may result in an increased risk of both short- and long-term developmental neurological, pulmonary, and visual dysfunctions (Dammann *et al*., 2005) thus it is likely that the second fetus benefitted from increased time in the womb, potentially accounting for its survival. When multifetal pregnancies are complicated by the immature birth of one fetus, delayed interval delivery may offer a greater survival chance and favourable outcome for the remaining fetuses (de Jong *et al*., 1995; Kalchebrenner *et al*., 1998; Zhang *et al*., 2004; Arabin and van Eyke, 2009).

Our account of asynchronous parturition of twins in a non-human primate is to our knowledge the first reported case in the literature. Though circumstantial, it seems that this phenomenon may be a potential adaptive reproductive strategy, benefitting the health of the delayed neonate and ensuring successful reproductive effort of the mother in both human and non-human primates.

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