Reproduction in Domestic Animals

Reprod Dom Anim **46**, e97–e101 (2011); doi: 10.1111/j.1439-0531.2010.01610.x
ISSN 0936-6768

Short Communication

Dystocia and Fetotomy Associated with Cerebral Aplasia in a Greater One-horned Rhinoceros (*Rhinoceros unicornis*)

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Contents

The captive greater one-horned rhinoceros population consists of 176 animals. Since 1971, a total of 226 calves were born into this captive population. However, 24% of the offspring born were either stillborn or did not survive the first 3 months. The causes for this high rate of stillbirth and neonate mortality have not yet been documented. Here, we report on the veterinary management of a dystocia and foetotomy resulting from a malpositioned greater one-horned rhinoceros foetus. The dead foetus presented with a forelimb flexed at the shoulder joint, with all other joints extended. The foetus was dissected into five parts and extracted during two anaesthesias on two consecutive days. The dam recovered fully and came into oestrous 31 days after surgery. Post-mortem and CT examination of the malformed foetal head revealed cranioschisis with cerebral aplasia and cerebellar hypoplasia. The cerebral aplasia presented here and in other recent cases suggests that neural tube defects and cranial malformations may be associated with more captive rhinoceros stillbirths than previously considered. Epidemiologic studies of these phenomena and possible nutritional deficiencies or hereditary defects are warranted.

Introduction

The Indian Rhinoceros or greater one-horned Rhinoceros (*Rhinoceros unicornis*) once inhabited many areas of Pakistan, India and Nepal to Myanmar and may even have roamed in China but their distribution has shrunk as a result of recent human influences. At present, this endangered species only exists in several protected areas of Northern India and Nepal, while a few individuals remain in Pakistan. As their numbers have recovered from fewer than 200 earlier in the 20th century to an estimated 2850 today, the greater one-horned rhinoceros symbolises one of two great success stories in rhinoceros conservation (Foose and van Strien 1997; Talukdar et al. 2008). However, this founding population of 200 animals presents a severe genetic bottleneck.

The captive population in Asia, North America and Europe, as part of the global conservation efforts, currently consists of 176 animals in 64 institutions (von Houwald 2008). This population is subject to intense genetic management. Breeding partners are matched to ensure that all founders are genetically represented and genetic diversity is maintained at a maximum. Since 1971, a total of 226 calves were born into the captive population. Since the 1980s an average of 11 calves were born each year, suggesting good reproduction for the greater one-horned rhinoceros in captivity. However, 24% of the offspring were either stillborn or did not survive the first 3 months, reducing the number of surviving calves to an average of eight animals per year (von Houwald 2008). Causes for the high rate of stillbirth or neonatal mortality in the greater one-horned rhinoceros are not documented in the literature. Maternal aggression may be one cause of neonatal mortality in captivity. Signs of labour are sometimes hard to recognize, possibly causing dystocia to go unnoticed and resulting in foetal death. In this report, we document the dystocia and stillbirth in a greater one-horned rhinoceros and its veterinary management by performing a foetotomy.

Dystocia and Foetotomy

Dystocia occurred in a 17-year-old greater one-horned rhinoceros kept at the Lisbon Zoo. The rhinoceros had three previous pregnancies that all ran to full term. All three previous calves, sired by the same male, were born alive but died within a few hours after birth, or at the age of 2 and 2.5 months, respectively. The cause of death were not documented, but at necropsy of the animal that lived 2 months revealed an ocular dermoid, and the animal that lived 2.5 months a gastric ulcer was noted (Bernardino et al. 2007; Fernandes, personal observation).

The current calf is the fourth from this dam and her second with a congenital defect. After a normal gestational length of 503 days, the onset of labour was defined by the rupture of foetal membranes. The parturition progress soon stagnated despite regular labour activity. By 31 h after the rupture of the foetal membranes no part of the foetus had yet been observed and a dystocia was assumed. For physical examination, the animal was anesthetized using 1.5 ml of Immobilon (Large Animal Immobilon®, containing 2.25 mg etorphine base HCl and 7.38 mg acepromazine base per ml and supplied by Vericore Ltd, UK), which was administered in the right flexor digitalis muscle by means of a blow pipe. Anaesthesia was maintained for 3.5 h with additional intra venous injections of 0.2 ml Immobilon® into the ear vein at intervals of 30–40 min based on increased respiratory rate and reaction of the female on tactile pain stimuli. To help maintain blood oxygen saturation, medical oxygen was administered into one nostril at a flow rate of 10 l per min. The animal remained in semi-lateral recumbency on its right side.

Once the animal was anaesthetized, 17 g of amoxicillin (Clamoxyl LA®; Laboratórios Pfizer, Lda, Portugal) were administered intramuscularly as a bacterial prophylaxis.

Upon vaginal palpation, the foetus was cranially and longitudinally presented in dorso-sacral position with flexion in the right carpal joint and flexion of the left shoulder joint with extension of the ipsilateral elbow and carpal joints. The foetal head had progressed until its muzzle was 10 cm cranial to the vulva. The foetus was fetid, and it was presumed it had been dead for over 24 h. Despite the use of lubricants and attempts to repel and reposition the foetus with traction using obstetrical the malposition could not be resolved. In absence of foetal life signs (movements, reflexes, pulse) and upon palpation of an abnormal soft tissue opening on top of the foetal skull, a foetotomy was performed. (i) Using a Tygussen-foetotome, the head was cut off and delivered though the birth canal by grasping the orbits, (ii) The right front leg was then repositioned and cut off. The cutting wire went through the distal diaphysis of the humerus, (iii) The left front leg was repositioned and cut off through the shoulder blade. Attempts to further deliver the foetal body now using a Krey-Schüttler hook failed, (iv) The cranial 10 cm of the thorax were removed (Fig. 1). Further attempts to extract the foetus by means of the Krey-Schüttler hook and simultaneous intravenous and intramuscular administrations of 10 and 20 IU of oxytocin, respectively, (Placentol®; Ceva Saúde Animal produtos Farmacêuticos e Imunológicos, Lda, Portugal) were unsuccessful. Because of the extended duration of the anaesthesia (~3.5 h) and the development of severe swelling of the birth canal it was decided to reverse the anaesthesia by means of 3 mg diprenorphine i.v. (Revivon®; Vericore Ltd. UK).

Fifty hours after the onset of labour the dam was anaesthetized for a second time. The remaining part of the carcass had advanced well into the birth canal. A lyophilized lubricant powder (J-lube; Jorgensen laboratories, Loveland, USA) was administered between the foetus and the birth canal. The powder absorbed mucosal fluids, and effectively lubricated the foetus. To prevent mucosal swelling during the extraction process naphazoline nitrate, an alpha agonist (80 ml, Privin®, Novartis, Munich, Germany) was applied on the mucosae of the cervix, the vagina and the vulva. The remainder of the carcass was now extracted by two people using one birth chain attached to the humerus, while one operator protected the mucosa from penetration by sharp bony parts (Fig. 1). No internal lesions in the vagina or cervix were found after extraction. Another 20 ml of naphazoline nitrate (20 ml) was applied on the mucosa of the cervix, vagina and vulva reducing the mucosal swelling to some degree. To further contract the uterus and expel remaining uterine fluids, 10 and 30 IU of oxytocin were injected intravenously and intramuscularly, resulting in uterine contraction within 20 min. The uterus was flushed with 80 l of a 0.09% saline solution (tap water mixed with kitchen salt), using a hand-operated aquarium pump. Ten foaming pessaries each containing 463.4 mg of oxytetracycline were placed into the uterus and vagina

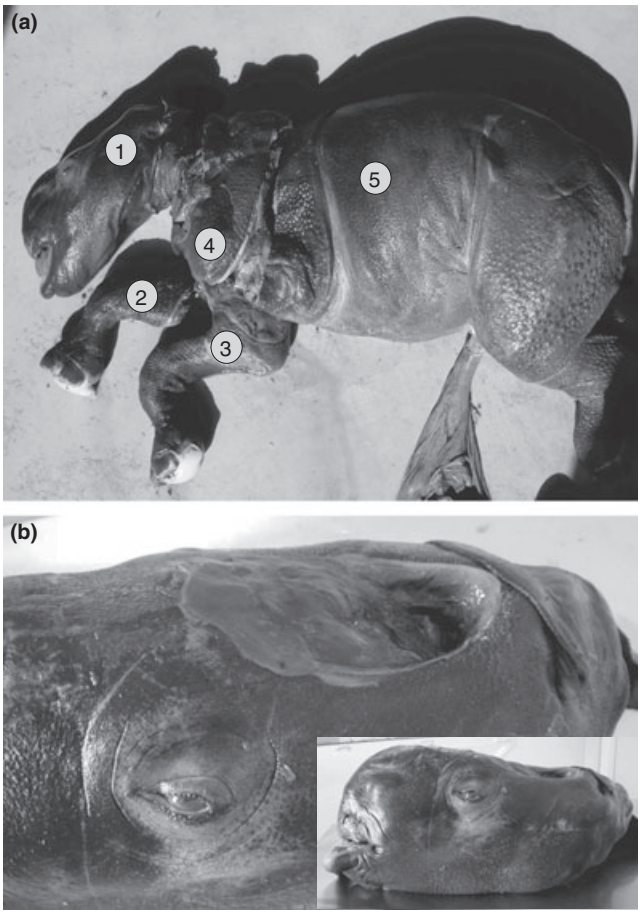

Fig. 1. (a) Stillborn greater-one-horned rhinoceros calf extracted in five parts by foetotomy. Numbers (1–5) represent the order in which parts were cut and extracted: head, right and left front leg, parts of the thorax, rest of the trunk (b) Cranioschisis: Absent skin and cranial roof allow direct access to remnants of the cerebrum

(Terramycin, Pfizer, Karlsruhe, Germany). The animal was treated orally twice daily for 14 days with 17g amoxicillin (Clamoxyl LA®). The anaesthesia was reversed using 2.5 ml naltrexone (50mg⁄ml; Naltrexone, ZooPharm, Fort Collins, USA). Recovery was uneventful. Post-partum oestrous was observed at 31 and 51 days after surgery. Transrectal ultrasound examination (Voluson I; GE Medical Healthcare, Munich, Germany, 2–5 MHz, 4–8 MHz probes) 100 day post-surgery confirmed complete uterine involution and showed active ovaries with one corpus luteum and multiple follicles. Minor scar tissue in the vagina with a spherical area measuring 0.3 cm were the only remnants of the surgical removal of the foetus.

CT of Cranial Malformation and Histopathology

To further investigate the noted abnormalities, the head of the stillborn calf and the head of a live-born, 4-week-old calf were compared using CT imaging (CT Lightspeed QXi, General Electric, Milwaukee, Wisconsin, USA; CT Aquilion One, Toshiba, Tokyo, Japan). The CT images demonstrated multiple congenital defects including: brachygnathism superior and cranioschisis

with cerebral aplasia (Fig. 2). CT-based virtual 3D reconstructions demonstrated the open roof of the skull palpated grossly. The paired parietal bones were almost completely lacking as were the caudomedial parts of the paired frontal bones. The edges of the resulting wide dorsal opening of the skull were formed by the frontal bones, by lateral branches of the frontal bones to which, presumably, lateral vestiges of the parietal bones had been fused laterally, and by the small paired interparietal bones which connected to the caudal occipital bone. The maxillary bones were shortened resulting in brachygnathism superior. The rostral view showed clockwise torsion of the maxilla, while the mandible had a counter-clockwise torsion. The number of teeth corresponded to that of the normal deciduous dentition: di 1/1, dc 0/1, dp (4/3), total 20.

Necropsy revealed no other lesions expect those of the brain and skull. Cerebral aplasia and severe cerebellar dysplasia with only the mesencephalon and medulla oblongata being fully developed were noted. The cranial bones and the skin had failed to fuse. At the rim of the opening, the meninx was tightly connected with the subcutis. In centre area, the dura mater was absent and the brain directly exposed. Both hemispheres of the cerebrum were marginally developed. The cerebellum was completely absent, but advanced autolysis prevented evaluation of development of any pons remnant. Only the brain stem was clearly identified and seemingly complete. The cranial part of spinal cord did not show any abnormalities. These findings classified the cerebral aplasia as cranioschisis, which was confirmed by histopathology. Only the brain stem and cranial spinal cord examined had no detected abnormalities.

Discussion

Stillbirth and neonatal mortality in captive greater one-horned rhinoceros occurs at an alarming rate of 24% of all births, but specific causes for stillbirths or neonatal mortality have not been reported. A report on a cyclopia (Ochs 2009), a personal observation of a cystic ocular dermoid (Fernandes, personal observation), and the cerebral aplasia presented here suggest that foetal malformation might represent an overlooked cause for the high incidence of stillbirth and neonate mortality in the greater one-horned rhinoceros. In spite of cerebral aplasia and cerebellar hypoplasia in the current case, the foetus was carried to term and was stillborn after 503 days of pregnancy. Congenital defects and neonatal mortality have been associated with genetic, environmental or unknown causes in a range of wildlife species (Leipold 1980). Yet, cerebral aplasia resulting from a

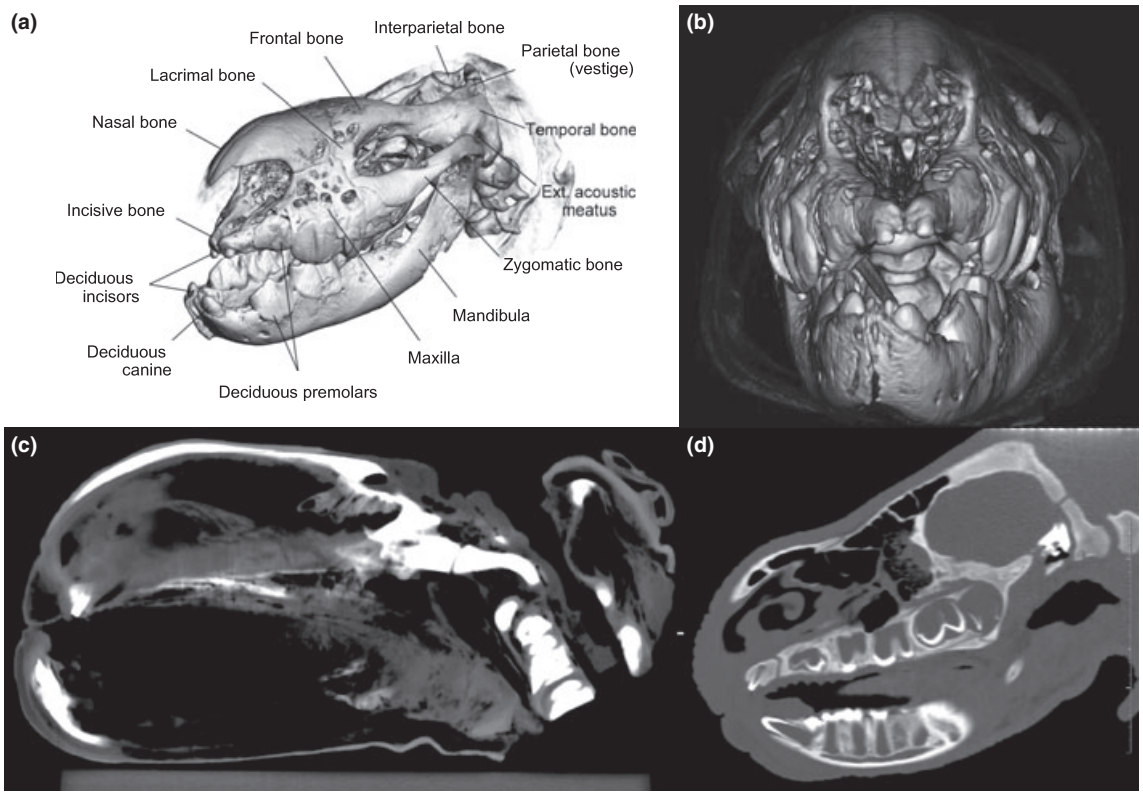

Fig. 2. (a–b): Three-dimensional CT images of a cranioschisis, brachygnathy and cerebral aplasia in a greater one-horned rhinoceros: a) rostrolateral view: note defect cranial roof, disproportionate lengths of maxilla and mandible. b) rostral view: asymmetric sagittal mid lines reveal the twisted axis of both the maxilla and the mandible. What appears as a foreign body crossing the oral cavity in this 3D perspective is the right stylohyoid. (c–d): Sagittal CT images of c) the stillborn and d) a live 4-week-old greater one-horned rhinoceros head. In both animals the sagittal plain runs through the incisors to demonstrate among others the axial torsion. In c), note the mandibular incisor rostral to the maxillary incisor, indicating brachygnathy; molar teeth not visible because of torsion of the head; elongated nasal bone; dorsally open cranium; an incomplete soft tissue shadow of the brain with absence of the cerebral hemispheres and cerebellum, and only parts of the brain stem present. In d), note the pneumatisation of dorsal skull bones, short bony nasal bone, molar teeth in the maxilla and mandible, and the closed, rostral pneumatised cranium with a soft tissue brain shadow

neural tube defect forming early during pregnancy has yet not been described in a wildlife species. The occurrence of three malformed, greater one-horned rhinoceroses within a period of 2 years suggests that malformations may be occurring with a high frequency in this small, captive population (n = 176), with an average of only 11 births per year. In the horse, the closest related domestic species to the rhinoceros, 4.6% of all abortions, stillbirths or neonatal deaths are linked to a congenital defect, but only 4.3% of these congenital defects are described as craniofacial malformations (Crowe and Swerczek 1985; Giles et al. 1993). The calculated incidence of craniofacial malformation in the horse is 0.2%, which seems much lower than 14% (three abnormal calves per 21 born over a 2-year period) in captive greater one-horned rhinoceros.

The dystocia resulted from malposition of the foetus. As in the elephant, caesarean section is not an option in the rhinoceros because of the thick integument, enormous intestinal weight and difficult wound management in unconditioned animals (Hermes et al., 2007). Though laparoscopic accessibility of the abdomen has been demonstrated in the rhinoceros (Radcliffe et al. 2000; Hermes et al. 2009b), a large incision, as is necessary to extract a 40–50-kg foetus, poses a great risk of post-surgical complications. In order to ensure survival of the dam, the preferred method of resolving a dystocia is to deliver the foetus via the birth canal.

Cerebral aplasia and cerebellar hypoplasia were diagnosed post-mortem and confirmed by histopathology. Although tissues or cells from the cerebellum were absent upon necropsy and histopathology, decomposition prevented full evaluation of the brain. Regardless, cranioschisis, cerebral aplasia and severe cerebellar dysplasia were documentable. Failure of fusion of the cranial bones and the skin further characterized this aplasia as cranioschisis. In addition, insufficient development of the dura mater suggests a meningocele; a primary defect that occurs as focal failure of dehiscence of the neural tube from the ectoderm (Maxie and Youssef 2007).

Although Akabane, bluetongue virus and mucosal disease virus, cause severe defects of the cerebellum and cerebrum in domestic species, none of these viruses cause failure of the neural tube closure (Charles 1994; Maxie and Youssef 2007; Vercauteren et al. 2008). The pathogenicity of these viruses is not known in the rhinoceros, but no outbreaks with these pathogens have been reported. The pathogenicity of these viruses is not known in the rhinoceros, but no outbreaks with these pathogens have been reported. A serosurvey did demonstrate antibodies to these viruses in free ranging, black (*Diceros bicornis*) and white rhinoceroses (*Ceratotherium simum*) (Fischer-Tenhagen et al. 2000). Screening for these pathogens still is recommended.

In humans, cerebral aplasia and true anencephaly occurs when the cephalic end of the neural tube fails to close, between the 23rd and 26th day of pregnancy. Ninety-five percent of human anencephalic foetuses get aborted. In the 1970s, one of 150 000 babies in North America was born with anencephaly per year. Fifty-five percent of babies with anencephaly carried to term were stillborn (Timson 1970). Those babies born alive never gain consciousness and die within hours or days after birth. The heredity of anencephaly in humans is not certain. Recent studies have shown that anencephaly and spina bifida, the most common neurotubal defects could be related to folic acid (vitamin B9) deficiency. Substitution of folic acid to the diet of women of childbearing age significantly reduced the incidence of neural tube defects (Oakley 2009). Serum concentrations of folic acid have not been reported for the greater one-horned rhinoceros. Folic acid supplementation in rhinoceros with a history of conception failure or a malformed foetus may be considered as a prophylaxis to prevent neural tube defects until further data on folic acid serum concentrations in the rhinoceros becomes available.

Chromosome abnormalities have been identified as a cause for infertility in the horse. Cytogenetic analysis has become an increasingly important clinical tool to identify chromosomal aberrations responsible for repeated early embryonic death and infertility in the mare. Technical advances in cytogenetics even permit the identification of chromosome-specific aberrations (Lear and Bailey 2008). In rhinoceros, fibroblast and lymphocyte cell culture visualizing chromosomes and chromosome banding has been described for all captive rhinoceros species (Wurster and Benirschke 1968; Hösli and Lang 1970; Houck et al. 1994). Chromosomal polymorphism and Robertsonian translocations have been reported, but these aberrations have not yet been interpreted as causes for reproductive failure in rhinoceroses (Houck et al. 1994). As the karyotype of the dam and sire was known to be normal parental chromosomal polymorphism or Robertsonian translocations with possible subsequent malformation of the offspring were excluded. Yet, chromosome polymorphism such as translocations, inversions, exchanges or deletions in stillborn or malformed foetuses should be considered and tested in the future.

Modern assisted reproduction in rhinoceros aims at improving reproduction by means of endocrine monitoring, fertility assessment, artificial insemination, cryopreservation or *in vitro* fertilization (Schwarzenberger et al. 2000; Stoops et al. 2004; Hermes et al. 2006, 2008, 2009b; Hildebrandt et al. 2007). These tools are directed towards improving reproduction rates. The study of the relation of stillbirth, associated congenital defects and their causes should be further investigated in this species.

Acknowledgements

The authors thank the staff of the Lisbon Zoo, Centre for Prenatal Diagnostic and Frau Karin Hönig for their passion and professional assistance and Brandon Menzies for his edit.

Conflict of interest

None of the authors have any conflict of interest to declare.

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Submitted: 4 Sep 2009; Accepted: 17 Feb 2010

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