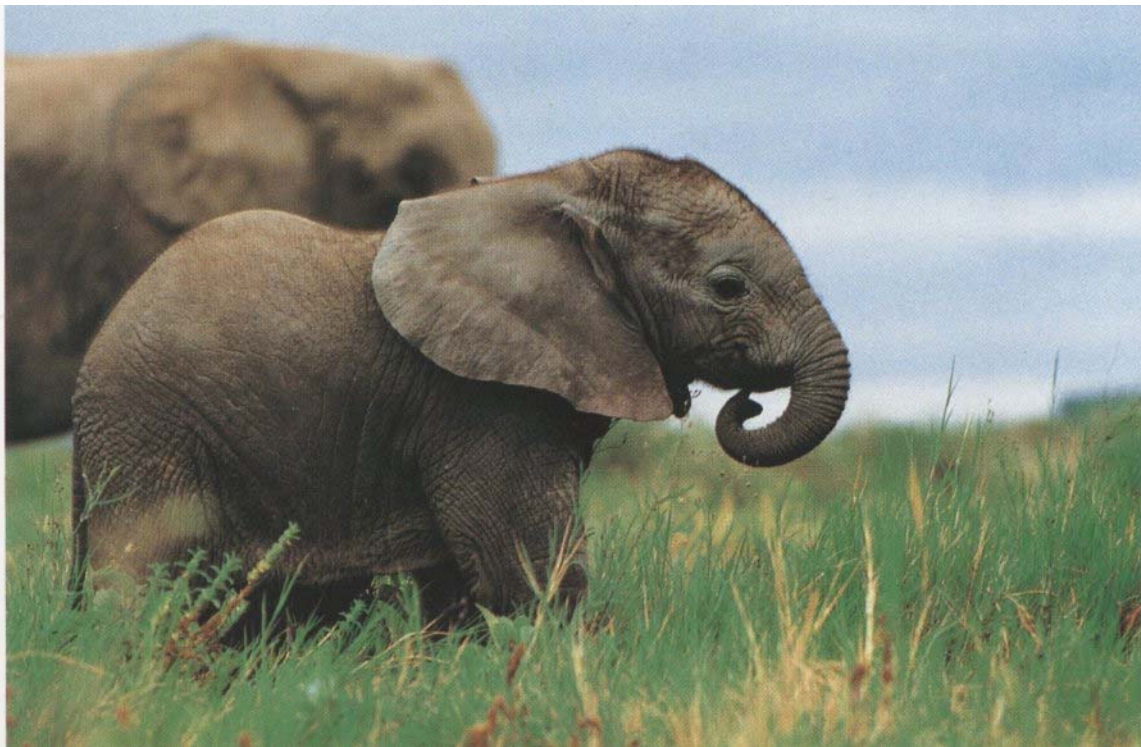


Disney's Animal Kingdom African Elephant Birth Protocols

Rev. 25 March 2005



African Elephant Birth Protocols Disney's Animal Kingdom

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PREPARATIONS

Equipment and Supplies

The following equipment and supplies will need to be on hand for the birth:

- 2 five-ton hoists or block and tackle assembly
- assortment of slings and belly bands
- harness for calf
- hand winch
- come-alongs
- portable light stands
- nylon and cotton rope
- pine shavings
- shepherd's crook/catch pole
- scale unit and platform
- platform, if needed, for nursing by calf
- towels
- medical supplies, as identified by Veterinary Team (see Appendix II and III)
- hand-rearing supplies and formula
- inner tubes (various sizes)—to be used for cushioning and support in the event of a full immobilization procedure
- veterinary supplies (see Appendix II)

Endocrine Monitoring

Blood samples will initially be obtained weekly for the purposes of monitoring progesterone. Twice weekly blood sampling will commence at Day 610 of gestation and daily AM blood sampling will commence at Day 627, or sooner, if there is a significant drop in P4 (defined as a 50% or greater drop from one sample to the next/and or if values have dropped to 5 ng/ml).

Daily samples will be run with the previous day's sample as a point of reference. When the progesterone value falls by half that of the previous value and is near or below 2 ng/ml, a PM sample may also be obtained and all three samples will be rerun to confirm that the P4 is at baseline.

Plasma

We will have equine hyperimmune plasma on hand for the following purposes: failure of passive transfer, sepsis, hypoproteinemia, and fluid supplementation. Plasma can be administered either IV or orally.

Although "natural" plasma from donor elephants is considered the preferred choice in some instances, equine hyperimmune plasma may be more effective/potent with a compromised calf. It has been used successfully here at DAK in several instances, is readily available, can be kept frozen here and is considered safe. The primary issues with using natural plasma are the separation/concentration process and storage.

Animal Training

All cows will be re-habituated to chaining. Pregnant cows will be on chains for parturition and it is anticipated that the other cows will need to be chained for their introductions to the calves. The amount of time that pregnant cows are on chains will be gradually increased until they are comfortable with overnight chaining. Overnight chaining will commence around Day 627 of gestation. Variations in the chaining procedures will be incorporated into the daily routine. Additional procedures will be added so that they become comfortable with palpations, teat manipulation and ultrasound (transrectal and transabdominal) in their stalls while on leg restraints. The goal of this training is for cows to remain tractable and calm for procedures, accept leg restraints on all four legs and tolerate overnight chaining prior to the actual birth.

Pregnant cows will be trained for teat manipulation as well as vulva/vestibule palpation (vulva/vestibule palpation based on the tolerance of the animal and the Zoological Manager's assessment of the safety of such a procedure in the stall). Cows will also be trained to perform a "step" behavior, in which they hold a front leg back to simulate a nursing posture.

Pregnant cows will be habituated to the presence of the scale and other supplies and equipment inside the stall while they are restrained on leg chains. They will also be acclimated to a nursing platform, in the event the calf is not tall enough to nurse without it. In the months prior to parturition, it may be necessary to occasionally hold a pregnant cow and one or more companions back from the group for periods of time to prepare them for separation during the actual labor and birth.

Ultrasound

Pregnant cows will undergo routine transrectal and transabdominal ultrasound examinations and assessments. These procedures will initially be conducted in the ERD and ultimately in Stall I while on leg restraints. The frequency of these ultrasound exams should be increased to weekly beginning at about Day 600 of gestation. Ultrasound procedures will be performed using the hand-held probe. There should be no need to use the extension probe around the time of parturition. The frequency with which ultrasound is performed closer to parturition will be based on hormonal data and progression (or lack of progression) of labor.

In addition to accustoming the cows to regular ultrasound procedures, these procedures will also serve in getting the elephants more comfortable with having members of the Veterinary Team present and interacting with them.

Once the pregnant cow's progesterone drops, or other signs of imminent labor present, the frequency of the ultrasound procedures may be increased to daily or even twice daily, as the situation dictates.

Birth Watches

Early monitoring will primarily be the responsibility of the Night Zoological Managers. Beginning at about Day 610, the Night Zoological Managers will begin surveillance on pregnant cows by checking them on remote video monitor three times a night and making informal notes on the dry/erase board outside the door. The Night Zoological Managers will have undergone a "birth training class" instructed by the Elephant Zoological Managers. This frequency will be increased to every two hours at Day 627. At that time, the Night Zoological Managers will be asked to make notations on the provided observation data checklist (see Appendix V). An Elephant

Manager will review the notes taken during the night the following morning and will be responsible for entering appropriate comments into the Daily Report System (DRS).

Night Keepers and/or Night Zoological Managers will begin continuous observations of the pregnant cows on or around Day 636 or when P4 values start to fall. At the same time, an Elephant Zoological Manager will begin staying overnight in the trailer. Once progesterone has dropped to baseline or upon observing other signs of impending birth, two members of the Elephant Team will be present at all times.

Monitoring will primarily be done from the tool room, via the use of a remote video monitor. The cow's behavior will be noted every fifteen minutes on a behavior data sheet designed to be suitable for presentation/publication purposes (see "First Occurrence," Appendix VI). So as not to disturb the elephants or disrupt the progression of labor, staff members will minimize movements around the barn and are discouraged from directly interacting with the elephants.

Observers will contact an Elephant Zoological Manager when any signs of impending birth are noted. It is the responsibility of that Manager to decide whether or not to mobilize the birth team. If the decision is made to mobilize the birth team, the communication procedures outlined in the section of this document "Communication Procedures and Contact List" will be followed (see Appendix I).

Video Documentation

The labor and parturition of the elephant calves will be recorded utilizing multiple stationary cameras as well as a hand-held video camera. Remote video monitors will be located in the upstairs Keeper office, in the tool room, and just outside the glass doors.

Practice Sessions

The entire birth team, including Animal Husbandry and Veterinary Team members, will assemble at the Elephant Barn on or around Day 600 for a "practice" session. This session will be used to:

- Clarify roles of all participants
- Visualize the barn set-up for the births
- Run through the locations and operation of cameras and monitors
- Simulate the pulling of the calf upon birth

Public Affairs Procedures

A meeting with representatives from Public Affairs and Media Relations will be held well in advance of the due date, to discuss the impending births and appropriate message points for all contingencies. A copy of the African Elephant Birth Protocol will be distributed to selected members of both Departments.

PRE-PARTURITION

Due Dates

Elephant gestation is reported to be approximately 659 days. Available data (n = 11) shows an average gestation of 648.5 days for African elephants (range 636-671 days), with a standard deviation of 10.4 days.

Vaccination of Cows Prior to Delivery

A tetanus toxoid booster should be delivered two months prior to the earliest due date.

Colostrum

Dripping of colostrum is not a reliable sign of impending parturition as this may occur several weeks before calving.

Progesterone Levels

- In general, progesterone levels will drop significantly 2-4 days prior to parturition (range 1-13 days).
- Daily blood samples to monitor progesterone will begin at about Day 627 of gestation.
- Although historically a drop below 200 pg/ml has been considered an indicator of impending parturition, we will not be using this value due to the complexity of our in-house assay.
 - Unlike most laboratories that measure one or a limited number of progestin (P4) compounds in their assay, our endocrine laboratory measures multiple progestin (P4) compounds with a resulting higher value measured in ng/ml. This has allowed us to monitor numerous species with the same assay.
 - As this is the first pregnant elephant that we have followed using this assay, the plan is to look at trends in P4 levels.
 - As parturition approaches and we gather more information on the pregnant dam including the level of P4 at which she plateaus, a more concrete numerical range may become available.
 - However, for now, any drop in P4 levels of 50% or more in a single day will be considered significant.

Cervical Mucus Plug

- Observing the cervical mucus plug is not the best indicator of impending parturition as it has been observed in only about 50% of births.
- When the cervical mucus plug has been observed, parturition has generally occurred within 12-24 hours.
- Although passage of the plug up to 14 days prior to parturition has been reported, we will consider lack of signs of labor 24 hours post-passage as an indicator for transrectal palpation and ultrasonography.
- Absence of labor 3-5 days post mucus plug passage favors a poor prognosis for delivery of a viable calf.

PARTURITION

It is important to note that pre-partum behavioral changes may be subtle. There have been “surprise” calvings with cows delivering both live and dead calves without any indication that birth was going to occur until the onset of active labor. Some elephants continue to eat right up to the time of labor and neither passage of the mucus plug nor dripping colostrum is noted until the onset of active labor. In the domestic horse, three stages of parturition exist with a distinct separation between them and a fairly constant timeframe recognized for each stage; the entire process from start of labor signs to passage of placenta usually lasts between 3-5 hours. In the elephant, however, stages 1 and 2 are somewhat hard to differentiate and times reported have varied greatly.

Stage 1: Labor

It is important to note that the signs listed below can occur in any order and may disappear and then return during the labor process.

- Subtle signs of labor include: restlessness, agitation, aggression, general discomfort, loss of appetite, abnormal posture, freezing in position, tail swishing, leaning into walls, rocking, placing the trunk in mouth, flapping ears more frequently, holding head down, crossing legs, shuffling, standing alone, and testing urine.
- More obvious signs of labor include: lying down and getting up frequently, kicking at abdomen, frequent defecation and/or urination, onset of colostrum dripping (see above comments), pulling teats, and swollen vulva.
- Marked signs of labor include: straining, passing mucous (see comments above), frequent stretching, obvious abdominal contractions, bending knees, vocalization, head stands, going down on one knee, squatting, bloody discharge, and development of a bulge beneath the tail.
 - The bulge should be considered to represent the fetal elephant in the birth canal and not simply the amniotic sac; the bulge will persist following amniotic sac rupture.
 - During the labor process, the bulge may disappear and reappear.
 - Most live calves have been delivered within one hour following appearance of the bulge (a few within 12 hours, one at four weeks).
 - This amniotic sac will usually rupture as the bulge increases in size, but occasionally does not until the calf is delivered.
 - Amniotic fluid should be large in volume and “clear”; differentiate from urine.
 - Once the amniotic sac has ruptured, the calf needs to be delivered within 12-24 hours to maximize its chance of being viable.
- Cessation of the labor process has been observed in > 50% live births. Any cessation of labor greater than 1/2 hour combined with a lack of progression of the calf down the birth canal will be followed by examination via transrectal palpation and ultrasonography. We may also be able to use a 3-m. endoscope to determine if the fetal membranes are intact or not. Due to staff safety reasons, this procedure would likely be conducted in the ERD.
 - If we wait too long to induce labor, there is a risk that the uterine muscles may become too tired.
 - It has been demonstrated in other births that the risk is in the waiting.
- The period of “hard labor” (characterized by the more marked signs described above) should last 20 minutes to 3-4 hours.
- The entire time from initial signs of parturition to delivery should be within 24 hours, with an average of approximately 10.7 hours. Approximately 10% of live elephant births

- have occurred with total labor times of > 24 hours with one delivery of an Asian calf at ~ 60 hours.
- The use of oxytocin and/or calcium gluconate for induction of parturition has only been required/used in ~3-4% of live births. Delivery usually occurs within 30 minutes after administration intravenously; repeated dosing of oxytocin following failure to deliver has had limited success. The administration of estradiol cyprinonate approximately one hour before oxytocin is given facilitates the softening of the cervix and the effects of the oxytocin. An attempt at rectal massage to induce contractions will be made prior to deciding to administer oxytocin or calcium gluconate.
 - Calf delivery has been approximately 50% posterior presentation and 50% anterior presentation.

Stage 2: Passage of Placental Membranes

- Passage of the placental membranes usually occurs within 4-5 hours of delivery of the calf (range 1-12 hours); oxytocin has been used to stimulate expulsion of membranes in only 4% of live births.
- The placenta and fetal membranes should be collected and delivered to the pathology department for weighing, examination, and sample collection (i.e. formalin, EM, frozen tissue bank).

Dystocia (“difficult birth”):

Dystocia has many causes. In elephants, most are felt to occur as the result of females with poor muscle tone, overly conditioned (obese) females, or excessively large or malpositioned calves.

- Breech births are easier with elephants. The most serious type of malposition is with the calf's head turned back.
- There may be a need to do a pseudo-episiotomy procedure in order to reposition the calf in the case of dystocia.
- If there is no apparent reason for lack of progress other than poor uterine contractions, medical intervention may be tried. Initial treatment with ECP aids in softening the cervix and producing mucus in addition to sensitizing the uterus to oxytocin. This should be administered approximately 1 hour prior to oxytocin. Oxytocin may be given IM or IV in progressive doses as long as active contractions and movement of the fetus in the birth canal is observed. Once the cow stops contracting, becomes fatigued, or the fetus appears “stuck”, drugs should be stopped.

The following signs may indicate that dystocia is present:

- Absence of labor 30 days past the known due date.
- Absence of labor 2-4 weeks after a significant decrease in serum progesterone levels. For our purposes, this is defined as a 50% or greater drop from one day to the next.
- No delivery 24 hours after the appearance of fetal fluids (i.e., rupture of the amniotic sac).
- No delivery several days after the appearance of a cervical plug. As mentioned above, lack of signs of labor 24 hours post-passage of the cervical plug will serve as an indicator for transrectal palpation and ultrasonography.
- Cessation of “active” labor combined with a lack of progression of the calf down the birth canal for an extended period of time. As mentioned above, any cessation of labor greater

than 30 minutes combined with a lack of progression of the calf down the birth canal will be followed by examination via transrectal palpation and ultrasonography.

- Lack of response to oxytocin.
- A fetid vaginal discharge +/- an increase in the systemic white blood cell count.
- A general deterioration in physical condition around the time of expected parturition.

Diagnosis/treatment/outcomes:

- Dystocia/malpositioning may be diagnosed with transrectal ultrasound, endoscopy and/or episiotomy.
- Episiotomy may be utilized to correct a dystocia, but may require a standing sedation or even a full immobilization to perform.
- If a fetus cannot be removed following episiotomy, but can be reached through the episiotomy site, attempts may be made to remove the calf via fetotomy once the calf is dead. This procedure would likely require a standing sedation or even a full immobilization to perform.
- There are no instances of successful C-sections; the failure rate is 100%. We will not attempt to do a C-section to retrieve either a potentially live calf or a dead calf.
- Retained dead fetus—there is one recorded instance of a mother dying due to a retained fetus; in addition, there is at least one case of a female with a retained fetus dying due to a ruptured uterus. In most cases, if the calf is dead and the amniotic sac is not broken, the calf will become dried out, mummified or calcified and will be delivered some months later with no ill effects to the dam. Consideration will be made to start the dam on appropriate antibiotics and serial blood work +/- vaginal cultures will be scheduled in the event of a retained fetus.
- If required to make a choice, we will always make a decision for the mother over the calf.

STAFF ASSIGNMENTS DURING PARTURITION

The presence of staff inside the barn during both the monitoring and the birth process will be minimized. Pre-parturition monitoring will be accomplished for the most part through video monitoring, so as not to disturb the elephants. When the birth team assembles at the Elephant Barn, only the Elephant Zoological Managers and the Elephant Keepers assigned to directly assist with the birth will initially be allowed on the main floor of the barn. No non-essential staff will be present in the elephant barn during the birth and immediately following, unless approved by the Curator or Animal Operations Director.

The compound will be “locked down” once the cow’s labor begins and only personnel with a true operational need will be admitted through the compound gate (staff required for the elephant birth as well as East Savannah staff working in the compound). The Security Kiosk at Compound F will be manned with a Security Host beginning on or around Day 636 of gestation. Backstage Safaris at the Elephant Barn will be suspended during the time of parturition and for a period of time afterwards, as determined by the Elephant Zoological Managers.

During the actual birth process, one Zoological Manager will be the primary handler for the pregnant cow, with a second Zoological Manager assisting. A third Zoological Manager will be the primary on pulling the calf as soon as it is on the ground and will be assisted by Keeper members of the “birth team” (total three Keepers).

The Curator and Animal Operations Director will also be present for the birth process to assist with decision-making and logistical support. Two Veterinarians, one Veterinary Technician and the three Keepers will stand by in the kitchen until their roles are called for. In addition to assisting with the pulling of the calf, the Keepers will primarily be responsible for recording the birth on a hand-held camera, making behavioral observations and monitoring the other elephants in the barn.

There will be two Keepers in the upstairs office monitoring and operating the camera systems and collecting data. These Keepers will be in radio communication with the staff on the barn floor, as needed. The balance of the Elephant Team and any other approved observers will be watching the barn activities outside the barn via remote video monitor. **There will be no opportunity to watch the birth from the upstairs balcony. This area will be off-limits.**

The pregnant cow will be housed in Stall I and will be on two leg chains for the actual birth, a left front leg chain and a right rear leg chain. It is at the Zoological Managers' discretion as to whether or not to leave the cow off chains until the "bulge" appears and then apply the two leg chains. Additional chains will be in place and available for use if necessary. We will have the ability to tighten rear leg chain(s) by attaching them to Gate 1 and using the hydraulic function of the gate to cinch them up as needed.

The calf will be pulled to a safe distance in the corner of Stall I closest to the kitchen, where the cow can smell and touch it but not grab or step on it. The scale and all exam supplies will have been set up in this location in advance. The calf will not be removed from the stall. At this time, the calf will be weighed and members of the Veterinary Team will enter the stall to perform an exam of the calf (see Neonate Evaluation below).

POST-PARTURITION

"First Occurrence"

A "first occurrence" study will be conducted to identify and monitor behavioral signs of impending parturition, document first occurrences of key calf developmental stages and to assess frequency of nursing in the first weeks of life. See Appendix VII for study details.

Neonate Evaluation

When calf is first separated from dam shortly after birth:

- Body weight: if possible also measure height, length, and girth
 - Average weight 232.2 lbs (105.5 kg) with a range of 117-330 lbs (53.2-150 kg); for average, high and low values were dropped.
 - Average height 35 inches (88.9 cm) with a range of 26-42 inches (66.0-106.7 cm); for average, high and low values were dropped.
 - (* Note: this data is based primarily on Asian elephants).
- Physical exam: complete systems evaluation including ophthalmic, oral, anogenital exams; thoracic and abdominal auscultation; conformation of legs; assess and treat umbilicus
- Blood collection: CBC, chemistry, protein electrophoresis, passive transfer tests (glutaraldehyde coagulation/zinc sulfate turbidity). Note: if blood is collected shortly after birth, it is expected that passive transfer tests will be negative.

- Herpesvirus PCR: whole blood, placenta sent to Dr. Laura Richman.
- Treat umbilicus with 30 ml tincture of iodine.

20-36 hours after birth:

- Body weight, if possible.
- Blood collection: CBC, chemistry, protein electrophoresis, passive transfer tests (glutaraldehyde coagulation/zinc sulfate turbidity). Note: passive transfer tests should be positive if the calf has nursed or received immunoglobins.
- Physical exam: repeat exam as above with special emphasis on oral exam and confirm passage of meconium through rectal exam.
 - Meconium is usually a yellow-orange mucilaginous material in the intestine of a full-term mammalian fetus and constitutes the first stool passed by the newborn.
 - Meconium should be passed within seven hours of delivery (92% of calves) and may occur almost immediately after birth.
 - Failure to pass meconium by two days of age should raise concerns about constipation, dehydration, and congenital anomalies.
 - The appearance of meconium during the parturition process is an indication of fetal distress.
- Treat umbilicus with 30 ml tincture of iodine.
- Herpesvirus PCR: whole blood sent to Dr. Laura Richman if possible.

Routine calf procedures:

- Umbilicus treatment: it is recommended that the umbilicus be treated with diluted Nolvasan solution QID for 1 day, then TID x 3 days. This may need to be accomplished while the calf is sleeping and/or the mother is restrained because it could cause pain or discomfort and distress to the calf.
- Body weight: daily or when possible during first 2 weeks; at least weekly during first year. Calf should gain approximately 1-3 pounds per day.
- Oral exam: twice daily (especially important during first 2 years). Look for ulcers or swollen, "blue" tongue. See Appendix IV for detailed information about Endothelial Inclusion Body Disease/Herpes Virus Infection in Elephants.
- Respiratory rate: twice daily (monitor trends); normal rate in 1 week old calf is approximately 20 breaths/minute.
- Heart rate: **ideally** twice daily (monitor trends); normal heart rates: 115 bpm (first week); 50-60 bpm (16 months of age).
- Rectal body temperature: **ideally** twice daily (monitor trends). Normal temperature: 97.5-99.0 ° F.
- Tetanus toxoid vaccination: 1 ml IM at 3 and 4 months of age; then annually
- Rabies vaccination: 2 ml IM at 6-8 months of age; then annually

Common medical problems in young elephants:

- Umbilical infections—the umbilicus may be very open at birth and there is potential for an ascending infection.
 - Diagnostic evaluation includes aerobic and anaerobic culture and sensitivity.
 - Treatment—topical cleansing and antiseptic flush; broad-spectrum antibiotic therapy.
- Diarrhea—occurs more commonly in hand-reared or formula-supplemented animals.
 - Changes in formula for hand-reared or supplemented animals are commonly used to manage mild diarrhea cases (diluting formula, discontinuing formula and substituting water, electrolyte solutions, rice water or rice milk, changing formulas, etc.).

- In cases of diarrhea accompanied by other clinical signs such as lethargy, weakness, reduced appetite, colic or dehydration, diagnostic evaluation as well as treatment are necessary.
- Clostridium, campylobacter, e. coli, pseudomonas.
- Constipation—has been reported in mother reared and hand-reared calves.
 - Can occur following a stressful event or abrupt diet change.
 - Signs include listlessness, anorexia, straining, absence of defecation, and rubbing hindquarters against walls.
- Metabolic bone disease—can occur when calves have imbalanced calcium:phosphorous ratios or chronic intestinal malabsorption.
- Herpesvirus—occurs mostly in young Asian calves (see section below on EIBD).
- Sunburn—calves are very susceptible to sunburn, especially on the head. Adequate shade must be provided at all times. Sunburn may be treated with a topical cream such as Vitamin E and restricted access to sunlight until healed.
- Heat exhaustion
- Skin dryness—can be mitigated by applying a mixture of lanolin and mineral oil
- Trauma—an infant that has been rejected may have received traumatic wounds

Evaluation of Dam

- “Lochia” is the normal discharge of fluid from the uterus following delivery of placental membranes.
 - It consists of normal and degenerate endothelial cells, blood, residual fetal fluids, and sloughed blood vessels.
 - This discharge should not be malodorous and can be present for up to two weeks in many species (uncertain duration in elephants).
- Weekly blood samples from the cow will be obtained for the first month post-partum as a means of assessing health and identifying potential problems early (CBC, chemscreen, fibrinogen, and EPH).
- Potential problems in cow:
 - Retained placental membranes (see comments above on normal passage).
 - Post-partum metritis (uterine infection). This usually manifests itself starting 10-14 days after delivery and may be mistaken for lochia initially, but is very malodorous in comparison.
 - Mastitis. This will be characterized by firm, hot, and painful teats and may be unilateral or bilateral. Affected cows may prevent the calf from nursing due to discomfort. Thick clots (potentially with blood) may be noted when milk is stripped from the teats.

Reintroduction to Dam

Once the neonatal exam is completed and if the initial assessment reveals no health problems, the staff will assist the calf in getting to its feet, keeping it close to the cow but out of harm’s way. At this time, the Veterinary Team will leave the stall and adjourn to the barn kitchen or the tool room.

Keepers and Managers will support the calf via the use of a rope or strap harness and prevent the calf from falling down as it attempts to walk. If the calf vocalizes or falls, the mother may become agitated or aggressive.

The calf should stand within 30 minutes of delivery (range five minutes to five hours). The time to standing that elapses beyond 30 minutes may indicate that tissue hypoxia (decreased oxygen delivery to cells), particularly brain tissue, and/or cerebral/cerebellar edema has occurred during delivery, most likely during passage through the pelvis. As time to standing increases, the prognosis for a normal calf worsens. For example, of five calves at the Oregon Zoo that did not rise to their feet within 30 minutes of delivery, only one survived to adulthood while four either died or were euthanized within two weeks.

Once standing, the calf will be reintroduced to the cow while she is still restrained. The calf will be fitted with a harness and tether so that it can be pulled back if necessary. The timing of the reintroduction will be dependent on the cow's behavior, the calf's ability to stand and motor steadily, and whether or not there are any medical issues with either dam or calf.

During the reintroduction phase, the cow will be assessed by the management team for signs of acceptance or rejection of the calf. As long as the cow is not overly fearful of or aggressive towards the calf, the calf will be brought closer to the cow. The behavior of the cow towards the calf will dictate the speed of the reintroduction process, which is intended to be gradual. As the cow becomes more comfortable with the calf moving around her, we will allow the calf more freedom to move behind and on either side of the cow. After the cow is comfortable with this, we will allow the calf to move in front of her. Once the cow accepts this, we will allow the calf to move under her abdomen. Keepers and Managers will station themselves on either side of the cow in order to "send" and "catch" the calf, so that its movements around the cow will be controlled.

Any affiliative or nurturing behavior on the cow's part will be rewarded. Any aggressive behavior towards the calf or staff members will be interrupted. We will essentially be "shaping" her behavior towards the calf.

Nursing

Once the cow is accustomed to the calf moving around and underneath her, the process of teaching the calf to nurse can begin. The calf will be guided to the cow's teat and allowed the opportunity to nurse while an Elephant Manager holds the cow steady. It may take the calf some time to become adept at nursing. As the cow and calf gain confidence and experience with one another, and as long as the cow does not show any aggression towards the calf, the Elephant Managers will determine whether or not to remove any of the leg restraints. This process could take anywhere from four to twenty hours to accomplish and is entirely dependant on the mother and her acceptance of the calf.

Attempts at suckling should occur within the first seven hours, but can take up to 24 hours. If the calf has not nursed within the first eight hours, discussion on the calf's condition between the Animal Husbandry and Veterinary Teams will be initiated. If no nursing has occurred by twelve hours of age, a decision will be made on whether or not to supplement the calf with milk stripped from its mother, colostrum, plasma, or formula. This decision will be made by the Animal Husbandry and Veterinary Teams in concert and will be based on many factors including: observations on the calf's vitality, strength of efforts to nurse, and assessment of dam's receptivity to the calf.

Approximately 74% of elephant calves attempt to nurse within seven hours of delivery (range 30 minutes to 24 hours reported). Of those calves that never nurse from the cow, approximately 50% do not survive. Of those calves that nurse, approximately 78% successfully nurse within

eight hours and 8% within 14 hours (range 1-10 days, with some animals obviously requiring some degree of supplementation).

If supplementation is required, the preferred route will be oral with the dam's colostrums and/or banked serum (commercial equine hyperimmune serum) utilized. Additionally, intravenous serum (commercial equine hyperimmune serum) may be administered to those calves that are felt will tolerate the procedure or are deemed weak and particularly at risk.

Once the mother is "trustworthy" with the calf, as determined by the Elephant Managers, all leg restraints will be removed and she will be allowed full movement around her stall with the calf. A continuous watch will be maintained on the dam and calf until we are confident that the mother is caring for the calf, that it is nursing well, and cannot injure it self or escape from the enclosure. The decision to discontinue the 24-hour watch will be made jointly by the Animal Husbandry and Veterinary Teams.

Introduction to Other Cows

A strategy for introducing the calf to the rest of the cows will be more finely developed after the birth has occurred and we are satisfied that the mother-calf bond is secure and that the calf is healthy and capable of keeping up with its mother. The Animal Husbandry Team will determine the introduction timeline, sequence and general steps:

- Initial introductions of cow and calf to the other cows will take place in one of the barn stalls or yards.
- The other cows will be introduced to the new baby one at a time.
- The calf may be tethered during the introductions to maintain the ability to pull it back if necessary.
- Two leg chains may be used to restrain a cow while the dam and calf are introduced.
- After the initial introduction to the baby and its mother, the restrained elephant may be released to further facilitate the introduction process, based on Zoological Manager assessment.
- Each introduction will be monitored by Zoological Managers and Keeper Staff and will be videotaped.

Socialization/Training of Calf

The calf will begin learning proper behavior around humans shortly after birth, with more formal training occurring as the calf develops. The calf will start learning not to engage in inappropriate/undesirable behaviors or behaviors potentially dangerous to the handlers.



AFRICAN ELEPHANT HAND-REARING PROTOCOL

General Notes

While a situation in which an elephant calf is mother-raised is obviously the optimal one, our institution will be prepared to hand-raise a calf or provide supplemental formula feedings if necessary.

In eleven reported cases of elephant calves being entirely hand-raised from birth, only five survived past infancy. Five other cases reported successful reintroduction to the dam after a period of up to ten days of bottle-feeding the calf.

Equipment and Supplies

- Commercially available bovine bottles (e.g., 2-pint "Little Giant" bottle)
- Commercially available bovine nipples with the openings enlarged for greater flow
- Formula
- Elephant plasma and colostrum if available

Colostrum and Plasma/Milking the Dam

In elephants, passive transfer of immunoglobins occurs via colostrum. Elephant calves are reported to consume between 2 and 10 liters of colostrum in early nursing bouts.

If possible, the dam should be milked so that the calf can receive natural colostrum. A cow can be hand-milked or a human breast pump can be used. Oxytocin can be given IM prior to milking attempts to facilitate milk let down. Milk yield can vary widely, from 300-1080 ml per milking. One zoo milked every three hours and used oxytocin each time, with average collections of 1080 ml per milking during the first week.

In cases of passive transfer failure, natural bovine colostrum can be obtained from certified Johne's-free dairies and utilized. Synthetic colostrum is also available.

Equine hyperimmune plasma has been used successfully at DAK in several instances, is readily available, can be kept frozen here and is considered safe. It may actually be more effective/potent with a compromised calf than "natural" plasma obtained from donor elephants. A supply of this equine plasma will be kept on hand and may be used with either a mother-raised or hand-raised calf as necessary.

Formula

There were a variety of formulas used in the sixteen hand-raising case studies that were reviewed, but the most successful and commonly used appeared to be the Grober African Elephant Calf Milk Replacer® formula. This product was formulated from analysis of milk collected from lactating female African elephants. The formula for African calves produces 750 Kcal/liter.

Nutricia Elephant Calf Milk Replacer® has been used in Israel and the Netherlands. Several human infant formulas have also been used, including SMA Goldcap/Gold® infant formula and Enfamil®. A soy-based formula, Prosobee®, was used in an Asian calf that proved to be lactose intolerant.

We will consult closely with our Nutritionist to develop a feeding strategy and/or make any adjustments to the formula, amounts and frequencies of feedings.

Feeding Amounts and Frequency

The number of feedings per day can vary from 5-9 per day, with 8-9 appropriate for newborns and 5-6 for calves approaching one year of age. One technique that has proved useful is positioning the calf in a corner and offering the bottle from behind. This seems to simulate a normal posture of the calf at the armpit of the dam.

Some general guidelines on amounts:

Calf Weight	Kcal per day	Volume of Grober formula
100 kg	6,000-8,000	8-10.7 L per day
200 kg	16,000-20,000	21.3-26.7 L per day

Calves should gain 1-3 pounds per day during the first year of life.

To date, the most successful hand-raising strategy for hand-raising African calves outside of Africa (Daphne Sheldrick's orphanage in Kenya), was the one employed at the Oakland Zoo in which a calf was bottle-raised from birth to 11 months of age, when the calf died from herpes. Consumption and body weight information over time for this calf are summarized in the table below.

Time Period	Avg liters/day (Grober's)	Weight of Calf (kg)
Week1	9.9	88.9
Week 2	11.6	91
Week 3	13.2	95
Week 4	12.0	98
Month 2	12.9	119
Month 3	16.2	136
Month 4	19.6	150
Month 5	21.3	158
Month 6	25.3	183
Month 7	24.6	208
Month 8	25.8	233
Month 9	28.5	246
Month 10	28.3	264
Month 11	30.6	294

Solid Foods

Calves will begin to experiment with solid foods at an early age. Small amounts of solid food, such as hay and other adult feeds, can be offered to a calf beginning at 1-2 months of age. Young calves can be encouraged to ingest small amounts of healthy, screened adult elephant feces on a fairly regular basis.

We will consult closely our Nutritionist regarding the types and amounts of any solids to be offered a calf.

Common Problems with Hand-Raised Calves

- Diarrhea—changes in formula are commonly used to manage mild diarrhea cases (diluting formula, discontinuing formula and substituting water, electrolyte solutions, rice water or rice milk, changing formulas, etc.)
- Clostridium, campylobacter, e. coli, pseudomonas
- Constipation
- Metabolic bone disease—can occur when calves have imbalanced calcium:phosphorous ratios or chronic intestinal malabsorption
- Herpesvirus—occurs mostly in young Asian calves
- Sunburn—calves are very susceptible to sunburn
- Heat exhaustion
- Skin dryness—can be mitigated by applying a mixture of lanolin and mineral oil
- Umbilical infection
- Trauma—an infant that has been rejected may have received traumatic wounds

The Veterinary and Animal Husbandry Teams will partner in assessing the health status of a hand-raised neonate and making any recommendations for courses of action.

Vaccinations

The Veterinary Team will determine the vaccination strategy and schedule.

Weaning

Very little information is available on the weaning age and process for hand-raised elephant calves. Mother-raised calves are weaned between the ages of four and five. However, as with ungulate species, weaning of hand-raised animals may occur at a much earlier age than mother-raised animals.

Below is a weaning strategy used for African elephant calves raised at the Sheldrick Wildlife Orphanage in Kenya.

- At 4-6 months of age, milled whole barley, oatmeal cereal and desiccated coconut are added to the formula (one tablespoon of each).
- The amounts are gradually increased until the formula has the consistency of porridge.
- At nine months of age, the calf should be receiving its largest volume of formula (about 28 liters per day).
- After this point, the amount of formula is gradually decreased. The calf should be picking up more solids at this time as well.
- At one year of age, the bottles are mixed with half formula and half skim milk. The cereal and coconut continue to be added.
- After two years of age, the formula and skim milk are gradually replaced with water, although the calf continues to be bottle-fed until the age of five years.

Appendix I. Communication Procedures

Night Birth

1. On-site observers contact "Night 1" or "Animal Care Duty Manager" by radio or telephone (321-228-1091). Call is made whenever any of the following signs of "hard" labor are observed:
 - Straining, frequent stretching, obvious abdominal contractions, bending knees, vocalization, head stands, going down on one knee, squatting, bloody discharge, or development of a bulge beneath the tail.
 - Note: staff should already be on alert based on progesterone levels and ultrasound indications of cervical dilation and/or movement of the calf down the birth canal.
2. ACDM first calls:
 - Matt Hohne
 - Jeff Bolling
 - Martin Ramirez
 - Tracey Dolphin
 - On-call Veterinarian
 - Dr. Don Neiffer
 - Dr. Michele Miller

Call home phone numbers first and then alpha page requesting a call back for confirmation. If there is no response to the alpha page, it will be followed up with a digital page with a call back number for confirmation.

The ACDM will communicate with the in-barn personnel as to who is en route to the Elephant Barn.

3. On-call Veterinarian calls the rest of the Veterinary Team.
4. Matt Hohne calls: John Lehnhardt, Elena Lamar, Mary Ellen Sheets, Tony Chapman (Chris Torge and Marcia Kayes are back-ups).
5. ACDM proceeds to call Cat Reburn and the balance of the Elephant Keeper Team. Note: all other Keepers will be notified and may elect to be present (unpaid), with the understanding that they will still be required to fulfill their regular shift requirements.
6. John Lehnhardt calls: Beth Stevens, Jackie Ogden and BUL Team Members.
7. Jackie Ogden calls Rena Callahan (Public Affairs). Call may be deferred until morning.

Day Birth

1. On-site observers inform available teammates (present in barn or via radio) and assign a teammate the role of making contacts. The call is made when any of the above signs of "hard" labor are observed. This person then assumes the role of "communications officer." Depending on the time of day and day of the week, the "communications officer" maybe able to delegate this function to a member of the Support Team (Paula or Doris).
2. "Communications officer" contacts:
 - Matt Hohne
 - Jeff Bolling
 - Martin Ramirez
 - Tracey Dolphin
 - On-call Veterinarian
 - Dr. Don Neiffer
 - Dr. Michele Miller

Contact may be made by radio or pager, using the alpha page system and requesting a call back for confirmation. If there is no response to the alpha page, it will be followed up with a digital page with a call back number for confirmation. The designated "communications officer" will communicate with the in-barn personnel as to who is en route to the Elephant Barn.

3. DAK Veterinarian calls the rest of the Veterinary Team.
4. Matt Hohne calls: John Lehnhardt, Elena Lamar, Mary Ellen Sheets, Tony Chapman (Chris Torge and Marcia Kayes are back-ups).
5. "Communications officer" proceeds to call Cat Reburn and the balance of the Elephant Keeper Team. Note: all other Keepers will be notified and may elect to be present (unpaid), with the understanding that they will still be required to fulfill their regular shift requirements.
6. John Lehnhardt calls: Beth Stevens, Jackie Ogden and BUL Team Members.
7. Jackie Ogden calls Rena Callahan (Public Affairs).

Appendix II. Veterinary Supplies

****Note--Most of these items will be in boxes labeled with contents**

Rectal exam/vaginal exam supplies

- OB sleeves
- OB lube- New un-open container (2)
- Nolvasan Scrub (1 gallon)
- Stainless steel bucket (medical grade)
- Rolled cotton (3 rolls)
- 4X4 gauze (6 packages)
- exam gloves (1 box each of small, medium, and large)
- Towels (10-12)
- Sonosite Ultrasound with probes
- Elephant ultrasound adapter handles

Calf-pulling Equipment/umbilical treatment

- OB sleeves (sterile) (1 dozen)
- Gigli Wire
- Gigli Handles
- Soft ropes (3-4)
- Head lamp
- Portable lights (elephant team to provide)
- Flash light
- Vestibulotomy cutting template-- PVC pipe (2 foot long, 2.5-3 inch diameter, smooth ends).
- Sterile gloves (6 ½, 7 ½ , 8; 6 pairs each)
- 4 Surgical gowns (2 extra large, 1 large, 1 medium)
- Surgical scrub sponges
- Sterile hand towels (4-5)
- Large animal surgical pack
- Scalpel blades (#20, 3#10; one box each)
- Suture 1-0, 0, 1 PDS and Gut (cutting and taper)
- 3" Spinal needles (20g, 18g; one box each)
- Lidocaine 2% (2 bottles – 100ml)
- Sterile drapes (multiple sizes, include paper and towel)
- 3 meter endoscope and associated equipment

Emergency drugs/supplies

- Portable suction unit
- Shop Vac with suction tip
- IV stand
- Lactated Ringers Solution – 5 Liters (4 bags)
- Lactated Ringers Solution - 1 Liter (5 bags)
- 0.9% Saline – 1 Liter (5 bags)
- 5% Dextrose in Saline – 1 Liter (5 bags)
- 50% Dextrose- 500 ml (2 bottles)
- Irrigation saline- 500 ml (5 bottles)
- Emergency Drug Box (See Appendix 2 and 3)
- Needles (14g, 16g, 18g, 20g, 22g 1.5", 23g, 25g; one box each)

- Syringes (1cc, 3cc, 6cc, 12cc, 20cc, 60cc luer-lok, 60cc catheter tip; one box each)
- ISTAT blood analyzer with cartridges

IV fluids/syringes/needles/miscellaneous supplies

- Lactated Ringers Solution – 5 Liters (3 bags)
- Lactated Ringers Solution - 1 Liter (4 bags)
- 0.9% Saline – 1 Liter (4 bags)
- 5% Dextrose in Saline – 1 Liter (4 bags)
- Irrigation saline- 500 ml (4 bottles)
- Sterile urine collection cups
- Stomach tube, pump, and bucket
- Duct Tape (one roll)
- Nolvasan Solution (1 gallon)
- Isopropyl alcohol (1 gallon)
- Betadyne Solution (1 gallon)
- Garbage bags
- 1 Sharps containers
- 2 extension cords
- Portable ECG
- Scale
- Breast pump

Catheter and Fluid line supplies:

- IV catheters (22g, 20g, 18g, 16g, 14g; multiple lengths)
- Butterfly catheters 19g (1 box)
- IV line set-up (equine/large animal)—3 complete sets
- IV line set-up (standard small animal) – 3 complete sets
- IV extension sets and injection caps
- Heparinized Saline
- Elasticon and Vetwrap (Multiple widths)
- Bandage scissors
- White tape (1 inch, 2 inch, 4 inch; 5 rolls each)

Neonate Exam and Intubation supplies:

- KY Jelly- 1 box
- Endotracheal tubes (8-12mm)
- Flexible light source for intubation
- Catheter type stylets for intubation
- Laryngoscope w/long blade
- Oral speculum (Baseball bat)
- Large cotton swabs
- Tie gauze
- Nitrofurazone Ointment
- Umbilical tape
- Large sterile hemostat to clamp umbilicus if necessary
- Thermometer (1)
- Stethoscope (1)
- Bandage scissors
- Bulb syringe
- Blood tubes (red, blue, EDTA, Heparin)
- Culturettes
- Thermometer (1)

- Stethoscope (1)
- Ophthalmoscope (1)
- Ophthalmoscope extra battery
- Sharpie markers
- Silver nitrate sticks (1 tube)
- Sharps container
- 50% Dextrose- 500 ml (1 bottles)
- Pulse oximeter

Calf tube feeding/colostrum collecting supplies

- Human breast pump
- Suction unit
- Feeding tubes
- 60cc Syringes (catheter tip; one box)
- Oral speculum
- Feeding tube pump

Appendix III. Elephant Emergency Drugs and Medications

Calf Treatments- Based on 225-275lb (102-125kg)

Respiratory Stimulants/Treatments	Dosages	Dose	Volume
Doxapram HCL (Dopram) 20mg/ml	0.4mg/kg	41-50mg	2-2.5ml
Aminophylline			
Furosemide 5% 50mg/ml	0.8mg/kg	82-100mg	1.6-2ml
Shock/Acidosis Therapy	Dosages	Dose	Volume
Solu-Delta Cortef 50mg/ml	0.2-0.8mg/kg	25-100mg	0.5-2ml
Sodium Bicarbonate 8.4% 1mEq/ml	0.5-1mEq/kg	51-125mEq/kg	51-125ml
Cardiovascular Stimulants	Dosage	Dose	Volume
Epinephrine 1:1000 1mg/ml	0.02-0.03mg/kg IV, IC or intratracheal	02.04-3.75mg	2.04-3.75ml (1mg/ml)
Calcium gluconate 23%	20mg/kg	2040-2500mg	8.9-10.9ml
Antiarrhythmics	Dosage	Dose	Volume
Atropine S.A. 0.54 mg/ml	0.015mg/kg	1.53-1.88mg	2.8 - 3.5cc
Lidocaine 2% 20mg/ml	1.16mg/kg	145mg	5.9-7.25ml
Antibiotics	Dosage	Dose	Volume
Ampicillin 200mg/ml	5mg/kg IM, BID	510-625mg	2.6-3.1ml
Procaine/Benzathine Penicillin 300,000IU/ml	25000 IU/kg IM, SID	284000 IU	9.5ml
Amoxicillin 500mg/capsule	11-20mg/kg PO, BID	1704 (15mg/kg)	3.5 capsules
Ceftiofur 50mg/ml	2.2mg/kg IM, SID	250mg	5ml
Amikacin 50mg/ml or 250mg/ml	3-5mg/kg IM, BID	568 mg	11.4ml (50mg/ml) or 2.3ml (250mg/ml)
Trimethoprim/Sulfamethoxazole	23mg/kg SID, PO	2346-2875mg	
Antivirals	Dosage	Dose	Volume
Famcyclovir	See previous Herpes information section for dose and regimen	Treatment may require upwards of 36,000mg drug	Given orally or per rectum
Analgesics	Dosage	Dose	Volume
Flunixin Meglumine 50mg/ml	1.1mg/kg	125mg	2.5ml
Other	Dosage	Dose	Volume
Kaopectate			200ml q2-3 hrs PO

Adult Elephant Treatments- Based on non-pregnant average body weight of 7750 lbs (3522kg)

Respiratory Stimulants	Dosages	Dose	Volume
Doxapram HCL (Dopram) 20mg/ml	0.4mg/kg	1480mg	74ml
Solu-Delta Cortef 50mg/ml	0.5mg/kg	1761mg	35ml
Furosemide 5% 50mg/ml	0.8mg/kg	2818mg	56ml
Sodium Bicarbonate 8.4% 1mEq/ml	0.5-1mEq/kg	1761-3522 mEq	1761-3522ml
Cardiovascular Stimulants	Dosage	Dose	Volume
Epinephrine 1:1000 1mg/ml	0.02-0.2mg/kg IV, IC or intratracheal	70.4-105mg	70.4-105ml
Calcium gluconate 23%	20mg/kg	70440mg	360ml
Antiarrhythmics	Dosage	Dose	Volume
Atropine L. A. 15mg/ml	0.015mg/kg	52.83mg	3.5ml
Lidocaine 2% 20mg/ml	1.16mg/kg	4085mg	204ml
Antibiotics	Dosage	Dose	Volume
Amoxicillin 500mg/capsule	11-20mg/kg PO, BID	70440	141 capsules
Ceftiofur 50mg/ml	2.2mg/kg IM, SID	7748.4mg	155ml
Amikacin 50mg/ml or 250mg/ml	3-5mg/kg IM, BID	1761 mg	352.2ml (50mg/ml) 70.4ml (250mg/ml)
Trimethoprim/Sulfamethoxazole 577mg/ml	23mg/kg SID, PO	81006mg	157ml
Hormones	Dosage	Dose	Volume
Estradiol cypionate (ECP)		30 mg IM	
Oxytocin 20 IU/ml		30 IU to 60 IU IM, IV	1.5-3ml
Analgesics	Dosage	Dose	Volume
Flunixin Meglumine 50mg/ml	1.1mg/kg	125mg	2.5ml
Ibuprofen 800mg/cap		4800 PO, SID	6 caps
Tranquilizers	Dosage	Dose	Volume
Azaperone		200-400mg	10-20ml

Appendix IV. ENDOTHELIAL INCLUSION BODY DISEASE/HERPES VIRUS INFECTION OF ELEPHANTS

Definition:

Endothelial inclusion body disease (EIBD) of elephants is a rapidly fatal syndrome affecting both Asian and African elephants and is caused by similar, but genetically distinct novel herpesviruses in each species.

Incidence of EIBD:

Since 1985, 18 cases of EIBD have been confirmed in North American Zoos with 16 Asian and 2 African elephants affected. In Europe (Germany, Switzerland, Netherlands), 10 cases of similar herpesvirus infections have been identified in Asian elephants with two additional cases from Israel. Infection has developed in elephants ranging from 1-42 yrs of age, although most have become infected under age 7 yrs. Of these younger cases, most have occurred around the time of weaning (16-24 months of age).

Of the 18 North American cases, only 2 have survived. All survivors were Asian calves under the age of 2 years that were treated with the anti-herpes drug famciclovir. Another Asian calf of similar age died during treatment.

Potential Effect on Captive Populations

Without treatment, all cases of EIBD in elephants have led to fatalities. Prior to successful treatment of an Asian calf in 1997, EIBD was responsible for the death of approximately 18% of all Asian elephants born in North America since 1983. While the impact on African elephants has been less (one calf and one adult), the potential for marked mortality exists with this species as well.

Etiologic Agents (The viruses):

EIBD is caused by two very similar, but genetically distinct endotheliotropic herpesvirus with one causing disease in each elephant species. This distinction has been made by utilizing polymerase chain reaction (PCR) and gene sequencing technology. PCR technology is used with increased frequency in disease investigations (e.g., salmonella testing in our elephant herd at DAK). These viruses are considered novel herpesviruses with unique immunogenicity such that cross reactivity with other herpesviruses has not been detected. This finding is significant since there was some initial concern that the development of EIBD may have been related to exposure to bovine herpesvirus 2.

Both viruses have been detected in healthy African elephants. The virus that has proven fatal to captive African elephants has been isolated from pulmonary lymphoid nodules from healthy wild African elephants. Why the virus has proven fatal in two captive animals remains unclear. The virus that has proven fatal to captive Asian elephants has been isolated from cutaneous (skin) papillomas of healthy captive African elephants and lymphoid patches from the distal vaginal tract of healthy wild African elephants. In the latter case, these vaginal lymphoid follicle-containing patches periodically become hyperplastic and occasionally ulcerate which may afford transmission of the virus to another host. No deaths from EIBD have been reported in wild elephants.

Currently, the existence of any elephant herpesviruses in wild Asian elephants is unknown. This fact, along with the isolation of both viruses from healthy African elephants suggests that Africans may serve as a potential reservoir of herpesviruses that can cause EIBD in both elephant species. Although direct proof of such transmission has not been established, the co-mingling of Asian and African elephants should be minimized. At institutions housing both species, care should be taken to avoid cross-contamination between barns via personnel and equipment.

Pathogenesis (What do the viruses do?):

The word “tropism” comes from the Greek word tropos (turn) and refers to the affinity or predilection that one object (usually animate) has for another (animate or inanimate). In the case of herpesviruses, most are epitheliotropic or have a predilection for epithelial cells. Target organs for these herpesviruses usually include skin, oral/urogenital mucosa, liver, adrenal glands, and the brain. In contrast, the viruses causing EIBD are endotheliotropic with a predilection for the capillary (smallest blood vessel) endothelial cells of the heart, liver, and tongue.

As mentioned, the absolute mode of transmission is uncertain at this time. However, based on lesions and the course of the disease, the proposed pathogenesis is as follows: once the elephant becomes viremic (circulating virus in the blood stream), ensuing viral replication occurs in the heart and leads to endothelial cell damage with resultant capillary leakage and severe intramyocardial (myocardia=heart muscle) hemorrhage and edema. This damage can lead to cardiac failure due to disruption of the electrical conduction system of the heart, alterations in heart function due to increased swelling of cardiac muscle, myocardial ischemia (compromised delivery of oxygenated blood to tissue) with necrosis, and/or metabolic (e.g. potassium, calcium, ATP) derangement. The tongue cyanosis (“blue tongue”) often noted might actually be the result of cardiac insufficiency and decreased blood delivery to the other organs of the body.

Clinical Signs:

EIBD has a rapid onset and progression. Animals have died within 1-7 days of the onset of clinical signs. Of the three animals that survived with treatment, clinical signs generally worsened for 1-2 days after the initiation of therapy and slowly dissipated over the course of 10-15 days.

In most cases the first sign is an acute onset of lethargy. Decreased appetite and water consumption, and mild signs of colic may or may not be present. In one of the successfully treated cases, decreased food and water consumption coincided with an increased sensitivity to touch in the area of the tusks. Consequently, the abnormal behavior was initially attributed to discomfort associated with tusk eruption.

In discussions among the zoo profession, a large amount of weight has been put on the development of a swollen and cyanotic “blue” tongue (cyanosis tends to progress from the tip caudally). However, this has not been seen in all cases or has developed several days after the onset of other clinical signs. Another potentially overlooked clinical sign is the development of ulcers in the oral and pharyngeal cavity. While the examination of the tongue is somewhat easy, visualization of the hard palate, gum lines, and back of the throat can be more difficult in a calf, particularly an animal that is not yet clinically ill. Due to the rapid progression and onset of EIBD, it is imperative to evaluate the oral/pharyngeal cavity twice daily for at least the first two years of life. During times of stress (social, disease, pregnancy), shedding of virus from latently infected individuals may occur and pose a risk for all naïve elephants in the barn. Consequently, it is also

recommended to routinely examine the oral cavity of all animals in the herd during the periparturient period through weaning.

A more obvious indicator that EIBD is present is the development of subcutaneous edema. This edema is usually bilateral in distribution and often affects the head (especially the face and proboscis), neck, thoracic limbs, and flanks. In addition, edema and discoloration may be noted in the ocular conjunctiva.

As edema/effusion can also develop in the respiratory system, regular measurements (2-3 times daily) of the respiratory rate should be made starting on day of birth, with special attention being paid to trends. The normal respiration rate in a one week old calf is about 20 breaths/minute; the adult rate is approximately 4-6 breaths/minute. Any marked change in rate or signs of increased respiratory effort should be reported.

Other vital signs should also be monitored twice daily because increases in heartrate and body temperature have been reported in cases of EIBD. The normal heartrate during week one of life is 115 bpm; 50-56 bpm in a 16 month old animal; and 25-35 bpm in an adult. Normal body temperature is 36-37 ° C. (97.5-99.0 ° F.) with temperatures greater than 38 ° C. (100 ° F.) considered to be elevated.

Defecation should be closely monitored including frequency, stool quantity, and stool consistency as colitis/enteritis have developed in at least one Asian calf during the course of the disease, presumably due to damage to endothelial cells of capillaries in the gastrointestinal system. However, colitis/enteritis secondary to medical treatment can not be ruled out.

Special attention should also be paid to body weight (measured daily or as frequently as possible), nursing behavior, urination, and overall activity/attitude.

Necropsy Findings:

The lesions of EIBD are nearly identical in both Asian and African elephants.

Gross findings typically include hydropericardium (free fluid in membranous sac around heart), along with extensive petechial (small) and ecchymotic (large) hemorrhages within all layers of the heart. In addition, petechial hemorrhages associated with mesenteric and serosal (external surface of organs) surfaces are diffusely scattered throughout the peritoneal cavity. Cyanosis of the tongue is sometimes present as is hepatomegaly (enlargement of the liver) and ulceration of the oropharynx and large intestine.

Histology correlates well with the gross findings and also demonstrates the presence of intranuclear viral inclusion bodies within the capillary endothelial cells of the heart, tongue, liver, and to a lesser extent, the intestinal tract. Electron microscopy readily demonstrates the presence of herpesvirus.

Diagnostic Tests:

- Complete Blood Count (CBC) - On presentation, affected animals often have an elevated white blood cell count (leukocytosis) with an absolute decrease in lymphocytes (lymphopenia). Occasional absolute monocytosis has been observed. Thrombocytopenia (decreased platelets) is usually present and anemia (decreased hematocrit, hemoglobin, and red blood cell count) is sometimes noted to varying degrees. As with clinical signs, the CBC profile may worsen for a few days even after the initiation of therapy. A follow-up CBC is important in tracking recovery or decline of the animal's condition.

- Serum Biochemical Analysis (SBA) - Some elephants with EIBD demonstrate hypoproteinemia although it is uncertain if this is due to decreased production due to hepatic (liver) compromise, increased loss due to increased capillary permeability, or a combination of factors. Other SBA abnormalities noted in some, but not all cases, include elevations in liver enzymes (LDH, AST, total bilirubin) and CPK due to injury/insult to the liver and muscle tissue, respectively. In addition, azotemia (elevated BUN and creatinine) has been seen in association with dehydration in one animal that clinically demonstrated decreased water consumption.
- Polymerase Chain Reaction (PCR) testing – This test is run on whole blood collected preferably in lithium heparin (see shipping information below) and is used to detect herpesvirus viremia. It is specific for the viruses that cause EIBD. In addition to diagnosis, PCR can be used to monitor response to treatment as the test will move from strong positive to weak positive and finally to negative as the viremia is cleared. A shift from a positive to negative test may take between 8-14 weeks (data limited at this time).

Treatment:

Because of the rapid onset and progression of the disease, treatment needs to be initiated based on clinical impression rather than absolute diagnosis because PCR confirmation may take 2-3 days.

Famciclovir--Although only four cases have been treated to date, the use of Famciclovir (Famvir, SmithKline Beecham Pharmaceuticals, Philadelphia, Pennsylvania 19101, USA) holds promise as 3 out of 4 Asian elephant calves treated survived. Famciclovir is an orally administered pro-drug of the anti-herpes agent penciclovir to which it is quickly biotransformed upon consumption by the patient. The plan is to keep enough of the drug for three days of treatment on hand at DAK with identification of additional quantities made at the time of parturition.

Dosing--No formal pharmacokinetic data exists, but is currently being investigated and may exist by the time our calves are born. Consequently, dosing will be based on that used successfully in previous cases in three calves under 2 yrs of age. Based on some limited data serum penciclovir levels measured from 97 ng/ml to 4,365 ng/ml in one animal and were considered comparable with known therapeutic levels described for humans.

Delivery of Medication

- Orally
 - Day 1: Famciclovir at 6.4 mg/kg PO TID; an initial loading dose of 12.8 mg/kg orally will be given.
 - Day 4-21: Famciclovir dose changed to 6.4 mg/kg PO BID.
- Per Rectum
 - Day 1-2 of treatment: if medicating the animal orally proves to be too difficult or impossible, Famciclovir can be administered per rectum as a paste at a dose of 10.6 mg/kg BID.
 - Day 3- 30 of treatment: Famciclovir administered per rectum as a paste at a dose of 6.7 mg/kg BID.

Other Treatments Used in Three Calves with EIBD Under 2 Years of Age

- Furosemide: (used in all cases)

- o 0.8mg/kg IM/PO BID x 5 days
- o 0.67 mg/kg IM SID x 9 days.
- Flunixin Meglumine (Banamine): (used in all cases)
 - o 0.88 mg/kg IM BID x 1 day, then 1.2 mg/kg PO BID x 2 days
 - o 0.67 mg/kg IM BID x 9 days.
- Antibiotics (used to some extent in all cases)
 - o Benzathine/Procaine penicillin-G (800IU/kg of each drug) IM q 48hrs x 3-4 t_x's
 - o SMZ-TMP @ 20 mg/kg PO SID (duration unknown)
 - o Ceftiofur @ 2.24 mg/kg IM SID (duration unknown)
 - o Ceftiofur @ 0.8 mg/kg IM SID x 11 days.
- Other drugs to consider
 - o B-complex @ 5 ml IM SID (duration unknown)
 - o Equine/bovine hyperimmune plasma, dam's serum, hetastarch, and oxyglobin.

Monitoring Schedule:

Blood:

If EIBD develops, samples will be taken daily for CBC/chem/serum/vitamin-mineral panels/penciclovir levels/Herpes PCR/banking through first week of treatment, and depending on progression, biweekly to weekly until further notice.

If the animal is healthy, samples will be taken for CBC/chem/serum/vitamin-mineral panels/banking weekly for the first month of life. Depending on health and progress of calf, the frequency will be modified following discussions with Veterinary and Animal Husbandry Teams.

Examination:

If EIBD develops, a veterinary physical examination will be performed twice daily during the first week of treatment and should coincide with the treatment schedule. At the end of one week, the frequency will be modified following discussions with Veterinary and Animal Husbandry Teams.

Regular measurements of vital signs, including respiratory rate (3 times/day), heart rate (2 times/day), and body temperature (2 times/day) are to be made starting on the first day that EIBD is suspected. (See section above for normal values).

Behavioral Observations

Please refer to section above under clinical signs for suggested behavioral parameters to monitor.

Laboratory (PCR) Information

- 1) Collect 1-3 cc whole blood (preferably into lithium heparin, but EDTA will do) and gently rock for one minute.
- 2) Using sterile techniques, transfer blood to cryopreservation tube.
- 3) Send via Fed Ex overnight on dry ice. Wrap blood tube in bubble wrap. Alternatively, store blood in -70 or -80 degree centigrade ultralow freezer until ready to ship.
- 4) Call Dr. Laura Richman before shipping samples at (410) 955-4471 (lab) or (301) 253-8723 (home answering machine), or Dr. Richard Montali at National Zoo (202) 673-4716.
- 5) Shipping address: Dr. Laura Richman, Johns Hopkins University, 725 N. Wolfe St, WBSB 314, Baltimore, MD 21205; (410) 955-4471.

References

1. Use of famciclovir for the treatment of endotheliotropic herpesvirus infections in Asian elephants (*Elephas maximus*). *J Zoo Wildl Med*; (2000);31;4:518-522
2. Clinical and pathological findings of a newly recognized disease of elephants caused by endotheliotropic herpesviruses. *J Wildl Dis*; (2000);36;1:1-12

Appendix VI. "First Occurrence" Study

A study on the early development of African elephant calves will be conducted for each of DAK's births. The purpose of the study will be to identify and monitor signs of impending parturition, document first occurrences of key developmental stages, and to observe and document frequency and duration of nursing bouts in our African elephant calves.

Seventy-two hours of continuous data will be collected post-partum on all occurrences and duration of suckling. Incidences of selected "first occurrence" behaviors will also be recorded *ad libitum*. At the conclusion of the first 72 hours, structured behavioral observations will be conducted four times a day for 30 minutes each session, using an instantaneous scan sampling technique. These observations will continue until the calf is at least one year of age.

The data collected will assist in documenting early developmental milestones for African elephants. It is anticipated that this information will provide elephant managers with tools to assist them in assessing calf health and in making appropriate animal management decisions.

The results of this study will be disseminated externally to elephant management personnel via publication in professional journals and/or presentation at zoological conferences.

Ethogram for Elephant First Occurrence Study

Mother	
First milk (expressed by staff)	
First milk (dripping on own)	
Onset of labor	
Appearance of bulge	
Amniotic fluids	
Time of birth	
Placenta delivered	
Mother orientates to calf	
Sensory exploration of calf	
Calf	
Attempt to right itself	
Assumes sternal position	
Attempt to stand	
Successful assisted standing	
Successful standing on own	
Introduction to dam	
Flap ears	First time calf flaps one or both ears (recorded as time start only)
Moves trunk	First time calf moves trunk (recorded as time start only)
First unassisted step	
Coordinated walk	
Attempt to nurse	
Successful nursing	
Defecation	

Urination	
Use of trunk	
Vocalization	
Drinking	
Mouthing objects	
Food in mouth	
Pool use	
Tooth eruption	
Interaction with animal other than dam	
Other interaction	Any first behavior observed that is not listed that seems significant in the development of the calf. Should be listed as time start with option of time stop depending on the behavior.

Ethogram for Elephant Calf Development Study

Active/Inactive	
Rest/Sleep—Standing	Standing with trunk tip resting against the ground.
Rest/Sleep—Lying Down	Lying down in a position varying from curled up to stretched out.
Locomotion	Any movement (walking, trotting, etc.) in a forward or backward direction.
Other	Any other behavior not described in ethogram.
Feeding	
Nursing	Successful nursing attempt where calf is seen suckling on nipple of its dam.
Provided Food	Chewing movements of provided food.
Grass/Plants	Chewing movements of grass/plants from the exhibit.
Drinking	Bringing water to the mouth with trunk or actively placing mouth directly to water source.
Enrichment	Chewing movements of provided enrichment (browse, produce, etc.).
Dirt/Sand/Gravel/Other Substrate	Chewing movements of dirt/gravel/sand/other substrate from the exhibit.
Feces	Chewing movements of feces from self or other elephant.
Social Interaction	
Aggressive	Aggressive behavior that involves contact between multiple animals. Includes attacks with trunk, mouth, body or legs; sparring, head butt, pushing, tusking. Does not include

	aggression to keepers.
Affiliative	Prosocial behaviors such as social play, trunk tangle, or caressing with another animal.
Exploration/Manipulation	
Exhibit Elements	Manipulating or moving the trunk, body, mouth or feet over exhibit elements.
Enrichment	Manipulating or moving the trunk, body, mouth or feet over provided enrichment items.
Self	Exploration of the body using the trunk.
Body Hygiene	
Water	Splashing water onto its bodies with its trunk or kicking water with their feet; immersing itself in body of water.
Dust Bathing	Coating its body in dirt (or other substrate) by rolling on ground or bringing dirt to the body with its trunk.
Contact	Animal is physically touching another animal. May be active contact such as play-fighting, or passive contact such as resting against another animal.
Proximity	Animal is within one body length from another animal. May be stationary or locomoting.