Hunny Pot's Two Faced Diagnosis

by

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Class of 2022

Clinicopathologic Conference

February 25th, 2022

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Introduction:

Congenital abnormalities are defined by aberrations which occur during an animal's gestational period and are present upon birth of the fetus.[8] There continues to be debate as to whether such aberrations are a result of environmental factors, twinning, or a mishap during embryogenesis, and there are altering opinions as to the pathophysiologic explanations for such abnormalities.[2, 4, 7, 8, 10-12, 14]

Classification and etymologic definition of abnormalities as described here are established in alignment with naming conventions used to define twinning where differentiation is made according to symmetry, site of fusion, and degree of duplication present.[1] Such conventions are somewhat universalized in human medicine where *parapagia* refers to twins united in parallel. In the case of this calf, a cephalic parapagia is present and further defined as a case of *diprosopia.* Diprosopia, the duplication of craniofacial structures upon a single body and head, is differentiated from dicephalus which is characterized by two entirely separate heads.[2] The case of Hunny Pot is classified as *symmetrical parapagus diprosopus tetrophthalmos* meaning there is duplication of the facial features, including four eyes, upon a single neck and body.

Diprosopia – which is an anomalous result of conjoined twinning – and other similar aberrations are not unique to cattle and have been reported in several other species. However, these types of inherited abnormalities are most common in cattle and buffalo,[12] relatively well reported in sheep and pigs, and are rare in goats, cats and horses.[4] In fact, conjoined twinning accounts for 2.2-10% of all congenital abnormalities in cattle.[2] With this in mind, however, diprosopia is still exceedingly rare with an incidence of 0.4% in humans and a likely similar

incidence in cattle.[1] Diprosopia is significantly less common in typical household pets and, in fact, is not reported in dogs.

History & Presentation:

Hunny Pot was a three-day old Black Baldy heifer calf weighing 28kg (62lb) that presented to Mississippi State University College of Veterinary Medicine's (MSU-CVM) Food Animal services on June 22nd, 2020. She was born to a multiparous dam, and her sire was a registered Limousin-Angus hybrid bull. She was found in the pasture at her dam's side following birth and there was no evidence of dystocia. There was no report of congenital abnormalities within the herd previously, and the pasture did not contain known toxic vegetation, which the referring veterinarian assessed for.

On presentation, Hunny Pot was bright, alert, and responsive. She was minimally ambulatory with support and was unable to nurse due to a cleft palate. She had a temperature of 101ºF, was tachycardic with heart rate of 124 beats per minute, and tachypneic with a respiratory rate of 56 breaths per minute. Her tachycardia and tachypnea were attributed to a combination of stress and elevated environmental temperature. Meconium staining was visible on her hind end. She had two noses, each with two patent nares, attached to their respective maxillae. Each maxilla had a hard palate which made up the lateral aspects of a severe cleft palate. One mandible was present centrally with a functional tongue and an appropriate number of lower incisors for her age. Two eyes were present on the lateral aspects of the head which were normal in appearance and apparently functional though both affected by moderate entropion and horizontal nystagmus. An additional globe was located on the dorsal aspect of the central oral cavity with eyelashes present on both the upper and lower palpaebrae. However, the globe was

severely ulcerated (likely due to a combination of exposure keratitis and contact with the tongue). A large flap of extra haired skin was present on the crown of the head with an open fontanel palpable centro-dorsally. The remainder of the physical exam was within normal limits and revealed no significant findings.

Hunny Pot was fed approximately one-half pint of milk replacer via orogastric tube and propped between two hay bales overnight to maintain a sternal position and prevent aspiration pneumonia or pressure sores. The following morning her mentation and physical status remained unchanged; however, she was highly resistant to a second tube feeding and it was withheld.

Diagnostic Approach:

Possible environmental etiologies for the congenital abnormalities were explored via history and diagnostic sampling as discussed later in this paper. A jugular catheter was placed using standard procedure in the left jugular vein and Hunny Pot was transported to radiology services. She was sedated using 0.1mg/kg of Xylazine and 5mg/kg of Ketamine, each dosed for a 150-pound calf, intravenously via the jugular catheter. Examination of Hunny Pot's entire body was performed via computed tomography with contrast. Findings revealed that the two separate nasal cavities joined to a common nasopharynx and the frontal sinuses were nearly absent. The most lateral maxillary bones contained normal dentition. There was malformation of the maxillary, incisive, nasal, basisphenoid, frontal, parietal, and temporal bones with several open fontanelles. The globe located at the dorsal aspect of the common oral cavity was revealed to be bi-globed with two optic nerves each traveling to respectively sided anomalous optic chiasms. There was severe malformation of the cerebrum with both enlarged and anomalous ventricles which extend into the fluid filled calvarium (*hydrocephalus*). The cerebellum was normal in appearance and

the remainder of the body was composed of expected and normal anatomy for a three-day old calf. Sedation was not reversed, and Hunny Pot was euthanized immediately following CT using 15mL of Euthasol. Her body was transferred to lab services for necropsy on June 24th, 2020.

Necropsy Findings:

Necropsy findings were consistent with physical exam and computed tomography results. The central eye located within the common oral cavity was composed of two fused globes within a single orbit with a rightward oriented nictitating membrane. Upper and lower eyelids of the aforementioned globes were absent and there was severe keratitis. The skin flap located on the crown of the skull was free of fluid and did not have direct communication with the brain.

Further characterization of the globes was achieved via histopathology. Each globe possessed one lens, separate vitreous, choroid, retina, and optic nerves as well as partially complete sclera except at the conjoined portion which also contained a common fused iris and ciliary body. Additionally, the globes shared a cornea and anterior chamber. Histologic identification and verification of the presence of four total globes is diagnostically indicative of a degree of craniofacial duplication, a condition referred to as *diprosopus tetrophthalmos*.

Pathophysiology:

Numerous differentials have been proposed as the etiologic causes of such abnormalities as seen in this case including environmental, genetic, and embryologic. Environmental causes such as exposure to toxins or radiation; hyperthermic conditions of the oviduct or uterus; altered blood supply to the fetus; deficiencies of folate, vitamin C, or riboflavin; and hormonal imbalances have all been investigated to some degree for their role in pathogenesis.[2, 4, 10, 12] In the

case of this calf there were no clearly defined environmental factors which may have played a role in the genesis of her abnormalities. Teratogenic vegetation such as *Veratrum californicum*, though known for creating cyclopia rather than diprosopia, was not known to grow in the pastures which her dam was kept. Infectious (viral or bacterial) pathogens of the dam have been probed as etiologic causes in the case of this calf. Viral agents, such a Bovine Diarrheal Virus (BVD) and Akbane virus have been studied in current literature as potential causes. In one case described, serum neutralizing antibodies of the calf and dam were positive for BVD-MD, however, virus isolation was negative.[7] BVD was eliminated as a potential etiology in Hunny Pot's case following negative virus isolation of ear notch samples, and no additional environmental factors could be verified to play a role in the calf's diprosopia.

The pathogenesis for diprosopia is unknown. Popular theories include involvement of genetics and divergence of the normal embryonic period. It is important to understand basics of the normal embryologic period before faults in the process can be investigated as possible explanations for abnormalities such as diprosopia. Under normal circumstances, the brain dominates cephalic development with later contribution from the digestive and respiratory tracts (i.e. nasal, laryngeal, esophageal, and tracheal structures). Neural progenitor cells develop within what is called the *neural crest* from where they migrate to various sites to differentiate further into several types of cells, including the facial bones. *Gastrulation* refers to the formation of the three germ layers from the epiblast: the ectoderm, mesoderm and endoderm. The *primitive streak* is the thickened layer of epiblast which establishes the cranial and caudal axis of the embryo and results in division of the embryo into left and right sides.[5] The streak eventually forms the *primitive node* which migrates further cranially to form a column of mesodermal cells referred to

as the *notochord*. The cluster of mesodermal cells cranial to the notochord creates the *prechordial plate*.[9]

Twinning under normal circumstances can be classified as either dizygotic - arising from two ova separately fertilized - or monozygotic - a single ovum fertilized by a single spermatozoon. Dizygotic twinning is the most common in cattle, particularly dairy cattle, resulting in conception of two separate calves. Monozygotic twinning may occur at different phases of development. The most common phase at which this occurs in cattle is the *two blastomere* stage where each blastomere gives rise to a separate individual. Alternatively, monozygotic twinning at the *inner cell mass* stage results in separate amnions with a shared yolk sac and chorion. Conjoined twinning results from the incomplete separation of two primitive streaks. The incidence of this in humans is 0.001% with a similar rate seen in cattle.[9, 14]

As mentioned, abnormalities of the embryologic life cycle have been proposed as etiologic explanations for diprosopia. With relation to conjoined twinning, three theories for the mechanism of development are studied: fusion, collision, and fission, with the latter being the most common explanation. Fission, or the incomplete separation of the inner cell mass, may result in abnormal embryonic axes at varying degrees.[2] Normally, this occurs prior to the 13th day following fertilization at which point differentiation of the embryonic disk takes place. Failure to do so yields shared body parts in addition to a common chorion and amnion[5, 11] seen with monozygotic twinning. *Diprosopus monauchenos*, where *monauchenos* refers to the presence of a single neck, could be explained by duplication of the notochord with the cranial part of the notochord being responsible for the duplication of cephalic structures.[4, 10, 14] As such, a bifurcated prechordial plate either due to fusion or incomplete fission is a possible cause of diprosopia.[2]

Diagnosis and Treatment:

As diprosopia is generally grossly apparent at birth, the diagnosis of such conditions should be focused on identifying congenital abnormalities during gestation. Given that the highest incidence of diprosopia is seen in cattle, early detection is of even greater importance as economic impact is significant. As such, diagnosis and treatment are one in the same, as the best treatment is prevention or early detection.

Prevention is best approached by managing herd breeding in a way that limits proposed environmental factors which may cause congenital abnormalities such as diprosopia. Removal of possible teratogenic plants from pastures and maintaining BVD, Bluetongue, and Akabane Virus free herds eliminates several pregnancy risks in addition to promoting general herd health. As in any calving operation, careful selection of breeding stock should occur as to optimize health and productivity of the progeny. However – assuming lack of other known genetic predispositions (i.e. vaginal prolapse) – a dam which produces a calf with diprosopia is unlikely to do so again and can be productively bred in the future.

Detection of congenital abnormalities in human medicine is largely based upon ultrasonographic studies and is essential for obstetrical management of these cases.[1] Pregnancy in cattle can be detected via transrectal palpation as early as 30 days of gestation. This is a reliable method for diagnosis of twinning if bilateral but not in unilateral (monozygotic) twinning as seen with cases resulting in parapagia. This conundrum can be bypassed using transrectal ultrasonography (TRUS) which is capable of detecting both bilateral and unilateral twins.[15] TRUS is most widely used amongst dairy cattle where accurate ultrasonographic detection of pregnancy can occur as early as 26 days following AI and twins identified by 40-55

days following AI.[6] The sensitivity and specificity of pregnancy diagnosis via TRUS are 97.7% and 87.7% respectively when conducted from 26-33 days post-AI, making TRUS an accurate and rapid method of detection.[6] However, one study showed a decreased efficiency in detection as compared to transrectal palpation when used in beef heifers at the end of a 108 day breeding season.[6] Other methods of detection include measurements of pregnancy proteins in serum where dams carrying two embryos were shown to have a higher concentration of PAG-1 (pregnancy associated glycoprotein). Unfortunately, this is not a widely available detection method and due to short half-life of PAG is not as reliable as TRUS.[15]

Management of identified cases of twinning, diprosopia, or other unfavorable congenital conditions may include culling, abortion, or continued management to parturition.[6] Continued management poses a significant economic impact as incidence of dystocia and stillbirth are greatly increased. Additionally, the severity of malformations present at birth in most cases of diprosopus not only preclude most calves from surviving, but also yield a nonproductive animal. For example, one of the most frequently associated anomalies with animals exhibiting diprosopia is a cleft palate,[8] as seen in the case of Hunny Pot, a condition which generally requires extreme intervention to provide adequate nutrition. Consequences of this defect may include inability to feed independently, failure of passive transfer and its sequelae, and aspiration pneumonia. Vigilant management of feeding via orogastric tube would be required for the duration of the animal's life. Though cleft palate repair has been documented[3, 13] its efficacy in prevention of severe complications remains negligible and no successful or cost-effective solution has yet been discovered.

Abortion may be selected to minimize cost and consequences of diprosopia. Manual embryo reduction is the most economically conducive option to mitigate negative periparturient

effects, though at present is not used commonly.[15] An alternative and more commonly used strategy is the use of prostaglandin F2alpha (PGF-2a) to induce abortion.[1, 15]

Conclusion:

Parapagia is exceedingly rare and nearly always carries an extremely grave prognosis. Though animals with diprosopia are typically affected to varying degrees, Hunny Pot's case was representative both of expected abnormalities as well as shortened lifespan. The pathogenesis of congenital duplication is poorly understood and continues to be debated. Current literature supports occurrence by either fusion or incomplete fission of the embryonic discs. Etiologies such as teratogenic vegetation, radiation, infectious pathogens, and maternal factors have been proposed as causes for diprosopia though further studies are required. Additionally, evidence exists supporting genetic and hereditary predispositions for such aberrations. While several diagnostic modalities are available, ultrasound is used most often and regarded as most accurate for detection of twinning and congenital duplication. Given the grave prognosis of affected fetuses and high incidence of dystocia, abortion and prevention of future affected offspring is the most economically effective approach to management of these cases. In contrast to the prognosis of the calf, that of the dam is excellent and future offspring are expected to be carried and delivered normally.

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