Overo Lethal White Foal Syndrome

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ABSTRACT: Overo lethal white syndrome is an autosomally inherited disease associated with horse breeds that register white coat patterns. The syndrome is associated with single amino acid substitution at residue 118 on the endothelin-B receptor gene and occurs in white foals born to American paint horses of overo lineage.8,9 The foals produced as a result of such breeding are known as lethal white foals and are born all white or mostly white. Although they appear normal at birth but fail to pass meconium and develop severe colic as a result of ileus caused by a functional intestinal obstruction. In the absence of veterinary intervention, death ensues, usually within 24 to 48 hours postpartum. Because there is no treatment for this condition, euthanasia is warranted to minimize unnecessary pain and suffering.

Overo lethal white syndrome is a fatal, autosomally inherited condition associated with white coat patterning in foals born to American paint horses (see box on page 0001–7) of overo lineage.8,9 The foals produced as a result of such breeding are known as lethal white foals and are born all white or mostly white. Although they appear normal at birth, they die or are euthanized shortly after birth because of myenteric aganglionosis in the caudal gastrointestinal tract, which leads to a fatal functional intestinal obstruction.10

Of the different subtypes of overo horses, lethal white foals occur most often in the frame overo subtype,4,8,11 although there is a report of an affected foal being produced from an overo–buckskin cross.9 Overo lethal white syndrome also occurs in miniature horses, half–Arabian horses, Thoroughbreds, and so-called cropout quarter horse foals that are born with too much white to be accepted into the breed’s registry. Similar conditions occur in rodents and humans, the most widely known of which is Hirschsprung disease in humans.12,13 Hirschsprung disease is also a congenital disorder characterized by aganglionosis in the distal gastrointestinal tract and is the most common obstructive motility disorder of the human colon, representing a cause of significant pediatric morbidity and mortality. Overo lethal white syndrome is considered by some to be a naturally occurring model for this human condition because it shares similar pathologic features with Hirschsprung disease, including endothelin–B receptor (EDNRB) mutation and nonfunctional segments of distal bowel.4 However, patients with Hirschsprung disease generally have normal melanocyte development, and this condition is not always fatal.

EMBRYOGENESIS

The pathogenesis of overo lethal white syndrome involves intestinal ganglion cells and melanocytes and results from a genetic defect involving neural crest cells during embryogenesis.4 The neural crest is a part of the folding neural tube that pinches off to form the cell bodies of all neurons and supporting cells outside the central nervous system, and conditions

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The Paint Breed

Coat Patterns
The coat color of paint horses may be a combination of white and any color appearing on horses. Coat markings vary in size and shape and can be located anywhere on a horse’s body. The American Paint Horse Association recognizes two distinct types of white coat pattern: overo and tobiano (A). The term *overo* comprises three different subtypes (i.e., frame overo, sabino, and splashed white) and is generally given to any paint horse that is not tobiano. The designation *overo* is derived from the Spanish word for speckled or egg-colored. Overo coloring is characterized by irregular white coloration on the abdomen that can extend to, but not cross, the dorsal midline between the withers and tail. White markings can vary from distinct regular patches to large irregular roan areas. The heads of overo horses usually have extensive white markings. One or more legs are usually dark, and the tail is usually one color. The frame subtype of overo coloration is called such because the white coat markings are framed by color. The sabino is an overo subtype with one or more white limbs and white facial markings, and the major characteristic feature is extensive roaning. Sabinos have irregular colored areas with flecks of white that blend with smaller white patches. The rarest overo subtype is the splashed white pattern in which horses have white legs, a white ventral abdomen, and a great deal of white on the head. Tobianos have body markings that are regular, distinct, and often in round or oval patterns. Tobianos predominantly have dark pigmented flanks, and all limbs are usually white. Head markings resemble those of a solid-colored horse, and the tail may be two colors. White patches are often oriented vertically and cross the dorsal midline. The American Paint Horse Association also registers horses as toveros (or tob-overos) when they have characteristics of both color patterns.

Genetics of Coat Color
Although the genetics of tobiano and overo coloration are not fully understood, some important factors have been established. The tobiano pattern is inherited as an autosomal dominant trait and has been mapped to a linkage group that contains albumin and vitamin D–binding protein. A polymorphism in intron 13 of the *c-kit proto-oncogene* (i.e., *KIT*) has recently been strongly associated with development of a tobiano coat. *KIT* encodes the mast cell growth factor receptor, a member of the tyrosine kinase receptor family, and is similarly associated with development of white spotting phenotypes in humans, pigs, and rodents. The *KIT* locus is linked to those that encode albumin and vitamin D–binding protein.

Genes that influence melanocyte development and migration have long been considered important in inheritance of an overo pattern, with the favored model being one in which overo horses are heterozygous for a dominant overo allele. Development of the frame overo phenotype is now known to be associated with a heterozygous mutation in *EDNRB*, the gene that encodes the endothelin-B receptor. However, there is variable expression of the frame phenotype, and some heterozygous individuals do not express the frame overo pattern, demonstrating the variable penetrance of the mutation. Nonframe phenotype heterozygotes may also arise because of fusion with other white patterns (e.g., tobiano, splashed white overo, calico overo, sabino overo) or the influence of other genes.

There is no association of this syndrome with an albino phenotype, which is a pigmentless white phenotype determined by a mutation in gene coding for the pigment–synthesizing enzyme tyrosinase.

such as overo lethal white syndrome arising from its defective development are called *neurocristopathies*. In vertebrates, both enteric ganglion cells and epidermal melanocytes arise from the neural crest.

After fusion of the neural folds and separation of the neural tube from overlying surface ectoderm, pluripo-
nervous system and pigment-producing melanocytes. Most of the neural crest cells migrate ventrally and emerge from beneath the somites to aggregate dorsolateral to the aorta, forming the sympathetic trunk ganglia. Other neural crest cells continue to migrate ventrally to form abdominal sympathetic ganglia and secretory cells of the adrenal medulla. Some neural crest cells cease to migrate when they contact the somites as well as form the segmental spinal ganglia, which contain sensory neurons. Cells derived from the neural crest region also form all accessory and glial cells in ganglia and Schwann cells that ensheath peripheral nerves. Another smaller group of neural crest cells, the melanoblast precursors, originate along the entire length of the neural crest and migrate through the dorsolateral pathway to colonize the skin and form melanocytes.

Two exceptions to this pattern of neural crest development exist. First, some neural crest cells originating from the sacral region of the spinal cord and the occipital region of the brain invade the gut wall, other internal organs, and blood vessels. Cells invading the gut wall form ganglia of the enteric plexus, whereas cells invading other internal organs form parasympathetic ganglia in various tissues throughout the body. The enteric nervous system precursors are predominantly derived from the vagal neural crest of the developing hindbrain and follow the ventral migratory pathway to enter the early embryo foregut and colonize the entire gut in a rostrocaudal progression. Second, some crest cells that arise from the cephalic neural folds have a broader range of derivatives and form facial connective and skeletal tissues as well as peripheral neurons of the head.

**Molecular Pathogenesis**

The EDNRB signaling pathway is critical in the development and terminal migration of neural crest cells that ultimately form melanocytes and enteric neurons of the enteric nervous system. EDNRB is a G protein–coupled, seven-transmembrane spanning protein, and endothelin-3 is one of its ligands. Overo lethal white syndrome in paint horses occurs as a result of substitution of lysine (Lys) for isoleucine (Ile) at residue 118 of the gene that encodes for EDNRB (i.e., EDNRB), and this mutation has been associated with the parental frame overo phenotype. Most solid-colored horses are homozygous for the Ile118 allele of EDNRB (wild type), whereas all parents of foals with overo lethal white syndrome foals are heterozygous for the Lys118 allele, and all affected foals are homozygous for this allele.

To produce a homozygous lethal white foal, two carriers of the mutated gene must be mated. According to Mendelian genetics, an overo–overo mating would be expected to produce 25% solid-colored foals, 50% overo foals, and 25% lethal white foals. However, analysis of stud book records and observation of foals born have demonstrated that the incidence of overo lethal white syndrome from overo breeding is much less than 25% and that some overo stallions never produce lethal white foals. In one small breeding trial, only six of 76 (7.9%) overo breedings produced lethal white foals.

**Clinical Presentation**

Affected foals are born with a white or almost all-white coat because of a lack of cutaneous melanocytes. These foals also have impaired innervation of the intestinal tract due to the absence of neural crest–derived submucosal and myenteric ganglia from the jejunum to the rectum. Because of aberrant embryogenesis and subsequent aganglionosis, the caudal intestinal tract, in particular, is underdeveloped and contracted, leading to neurogenic functional obstruction. Although these foals (Figure 1) appear normal at birth, they are unable to move ingesta distally along their intestinal tract, and once they begin to obtain colostrum and milk from the mare, they develop clinical signs of severe colic due to paralytic ileus. In most foals, these clinical signs are evident within 12 hours after birth and progressively
worsen. Without veterinary intervention, affected animals develop progressive abdominal distention and become increasingly painful. Intestinal rupture and peritonitis may occur as a consequence of paralytic ileus, and death occurs, usually within 48 hours of birth. In addition to the lack of cutaneous melanocytes and the presence of intestinal lesions, some lethal white foals are deaf, and many appear to have blue eyes because of the paucity of dark pigment on the posterior aspect of the iris.4,8,11

DIFFERENTIAL DIAGNOSIS

It is important to note that not every white foal has overo lethal white syndrome and that other diagnoses should be considered in young foals with acute abdominal pain. Two common causes of colic in newborn foals that may present similar to overo lethal white syndrome are failure to pass meconium and atresia of the distal aspect of the intestinal tract. Foals with meconium retention and impaction usually show signs of colic within 6 to 24 hours of birth, and this can often be diagnosed by digital rectal examination because most impactions occur in the distal aspect of intestinal tract. These impactions are also usually relieved via administration of an enema. Congenital intestinal atresia is an important cause of colic to consider in neonatal foals, and the absence of fecal material on the anus or perineum or the detection of clear, clean mucus following digital rectal examination is highly suggestive of this condition. Atresia ani can be easily detected by external examination of the perineal region of the foal, and the most consistent finding at the physical examination of foals with atresia coli is the absence of meconium staining after repeated enemas.

Although uroperitoneum due to urinary bladder or urachal rupture can also produce colic in neonatal foals, affected animals are usually dysuric, azotemic, hyponatremic, hypochloremic, hyperkalemic, and acidotic, which are findings that would not be expected in a lethal white foal. Other causes of colic in young foals (e.g., foal diarrhea or enteritis, small intestinal volvulus, intussusception, gastroduodenal ulceration) are less likely to be confused with overo lethal white syndrome because they mostly do not occur in newborn foals.

DIAGNOSIS

There is no definitive antemortem test to detect overo lethal white syndrome quickly enough to be of clinical value. Although exploratory laparotomy provides the most accurate means of making an immediate definitive diagnosis, this is rarely used as a diagnostic tool because a presumptive clinical diagnosis is usually considered sufficient.

The major factors to consider in making such a presumptive diagnosis include signalment and clinical signs as well as exclusion of other common causes of neonatal colic such as meconium retention and congenital intestinal atresia. Although not every white foal has overo lethal white syndrome, the possibility of this condition must be highly suspected in foals that are white or are the offspring of overo–overo breeding and have signs of colic and abdominal distention in the first 24 hours of life. Reduced intestinal and peristaltic sounds during abdominal auscultation are characteristic of decreased motility consistent with ileus and are suggestive of this syndrome. The possibility of urinary bladder or urachal rupture may be ruled out by the absence of serum chemistry abnormalities consistent with uroperitoneum, and digital rectal examination and response to

This is a fatal syndrome with no known successful treatment options.
enema administration help exclude atresia of the distal intestinal tract and meconium impaction. In foals in which the occurrence of intestinal atresia and meconium retention are excluded or passage of a meconium plug fails to relieve abdominal pain and the foal becomes progressively distended and painful, a clinical presumptive diagnosis of overo lethal white syndrome must be made.

**TREATMENT**
There are no successful treatment options for lethal white foals, and although attempts at interventional surgical resection of affected segments of the intestinal tract have been documented, such efforts have been unsuccessful because of the extensive nature of this lesion. Because of the lack of treatment success, this syndrome is considered lethal in all affected foals and prompt euthanasia is recommended when a clinical diagnosis of overo lethal white syndrome is made.

**ASSOCIATED ALLELE AND GENETIC TESTING**
In heterozygotes, the Ile118Lys EDNRB mutation is usually responsible for the frame overo phenotype, whereas this mutation in homozygotes causes overo lethal white syndrome. Because there is no treatment for this condition, identifying carriers of the lethal gene is essential for preventing or reducing its occurrence. Before genetic testing was available, carriers were identified phenotypically, according to the proportion of white in the coat; increased amounts of white were correlated with a greater risk of being a carrier. However, this technique is inaccurate because the frame coat pattern can be combined with other white patterns, making precise estimation of the EDNRB genotype difficult by visual examination of phenotypes.

The only way to determine with certainty whether white-patterned horses can produce an overo lethal white foal is by identifying the EDNRB genotype with a DNA-based test (currently available at the Veterinary Genetics Laboratory, School of Veterinary Medicine, University of California, Davis). DNA is extracted from the tissue sample, and the allele-specific polymerase chain reaction test locates and amplifies the specific mutated site in the EDNRB gene, thereby identifying horses that are heterozygous for the overo lethal white gene.

Hair or blood samples are routinely used for this procedure, and test accuracy is equal with either tissue type because all sources of DNA from an individual animal should give the same result. A sample of 30 to 50 hairs is required. The hair must include the roots and should not be cut (intact follicles are needed for testing because they represent the richest source of cells and hence DNA in the hair). Specimens should be collected from a clean region of the coat; laboratories recommend locating coarse hairs on the mane or tail, holding them close to the skin, and pulling to remove them. Hair samples should then be placed in a sealed plastic bag or envelope, taking care not to cross-contaminate the samples with hairs from other animals.

Whole blood is required for the procedure and must be submitted in either EDTA or heparin anticoagulant. A sample volume of 5 to 10 ml should be collected and delivered to the laboratory within 24 hours; refrigeration is necessary if there will be a delay between collection and submission. Because erythrocytes are anucleated, the test uses leukocytes as a source to extract genomic DNA from their nuclei.

The current cost of the test is $50, and 2 weeks should be allowed to receive results. Therefore, although DNA testing can definitively identify foals that are homozygous for the lethal white gene, results are not available soon enough for this test to be of practical clinical use in making an antemortem definitive diagnosis of this syndrome.

**POSTMORTEM FINDINGS**
Gross pathologic findings vary, but there is frequently marked gas and fluid distention of more proximal regions of the intestinal tract. Regions further distal have a narrow lumen diameter and lack ingesta, and the small colon is typically small and tightly contracted. Colonic stenosis and rectal atresia have also been reported in affected foals. Microscopically, myenteric and submucosal neuronal plexuses have reportedly been absent throughout regions of the intestine, and there is a lack of melanin in the skin.
CONCLUSION
Foals with overo lethal white syndrome are totally, or almost totally, white and, if not euthanized, die within days of birth from complications of intestinal aganglionosis. Although this condition is fatal, it must be remembered that not all white foals born of paint horses are affected. Consequently, such foals may not have aganglionosis and should not be euthanized at birth unless they develop signs of severe colic or are clinically diagnosed as lethal white foals. Foals that have signs of colic must be examined carefully to differentiate between this fatal syndrome and conditions such as meconium impaction or atresia of the distal intestinal tract. Because this syndrome cannot be treated, affected foals must be euthanized, and genetic testing is therefore essential to prevent its occurrence. Paint horses can be tested for the allele associated with this condition, allowing positive identification of breeding stock animals that are carriers of the lethal gene. Breeders can then avoid mating such carriers and locate new pedigree sources, subsequently breeding overo animals to only genetically proven non-overos. This genetic information can significantly help horse breeders prevent the emotional and economic effects associated with overo lethal white syndrome.

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REFERENCES

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1. Overo lethal white syndrome most commonly occurs in _________ foals.
   a. Arabian
   b. quarter horse
   c. American paint
   d. Thoroughbred

2. The underlying mutation responsible for overo lethal white syndrome involves
   a. EDNRB.
   b. c-kit.
   c. c-sis.
   d. the transforming growth factor–β receptor.
3. Overo lethal white syndrome is a model for which human disorder?
   a. Caroli’s disease  
   b. Fanconi syndrome  
   c. Hirschsprung disease  
   d. Sjögren’s syndrome

4. Which statement regarding overo lethal white syndrome is incorrect?
   a. The condition is fatal.  
   b. The melanocyte number is normal in the skin of affected foals.  
   c. Foals appear normal at birth.  
   d. Myenteric ganglia are absent or extremely reduced in number.

5. Most foals with overo lethal white syndrome die as a result of
   a. functional intestinal obstruction.  
   b. aspiration pneumonia.  
   c. metabolic acidosis.  
   d. acute renal failure.

6. The mutation associated with overo lethal white syndrome results in
   a. a functional gain.  
   b. single amino acid substitution.  
   c. chromosomal loss.  
   d. enzyme deficiency.

7. During embryogenesis, melanocytes and cells of the peripheral nervous system arise from
   a. myotomes.  
   b. branchial arches.  
   c. the neural crest.  
   d. the otic vesicle.

8. Which has(ve) been documented in foals with overo lethal white syndrome?
   a. cutaneous pigmentary defects  
   b. blue eyes  
   c. deafness  
   d. all of the above

9. The absence of meconium staining following repeated enemas in a foal with colic is most consistent with a diagnosis of
   a. atresia coli.  
   b. foal enteritis.  
   c. gastroduodenal ulceration.  
   d. uroperitoneum.

10. Which cell/tissue type is not derived from the neural crest?
   a. Schwann cells  
   b. enteric ganglia  
   c. melanocytes  
   d. teeth