The Impact of the Mutation Causing Overo Lethal White Syndrome on White Patterning in Horses

Paul D. Vrotsos, BS, CVT; Elizabeth M. Santschi, DVM, Diplomate ACVS; and James R. Mickelson, PhD

The mutation responsible for Overo Lethal White Syndrome usually results in frame overo white patterning in carriers. In 10–20% of carriers, this phenotype is unapparent due to suppression by other color genes or because of fusion with additional white patterning genes. To be certain of genotype in individuals, allele-specific PCR analysis must be performed. Breeders can use the genotype information when planning matings to eliminate the possibility of producing a lethal white foal. Authors' addresses: Peterson and Smith Equine Hospital, 4747 SW 60th Ave, Ocala, FL, 34474 (Vrotsos); Department of Surgical Sciences, University of Wisconsin, School of Veterinary Medicine, 2015 Linden Drive West, Madison, WI, 53706 (Santschi); and Department of Veterinary Pathobiology, University of Minnesota, 1988 Fitch Ave, St. Paul, MN, 55108 (Mickelson).

1. Introduction

White patterning (large white patches on the body and limbs) is a desirable trait in American Paint Horses, Pintos, Appaloosas, and Miniature Horses. It is an undesirable trait in the American Quarter Horse. American Paint horses have irregular, asymmetric white coat color patches on the body that are distinct from the small, symmetrical white spotting characteristic of the Appaloosa. The two main types of white patterning recognized by the American Paint Horse Association (APHA) are overo and tobiano.[1] Overo horses are characterized by white coloration on the abdomen that can extend to, but does not cross, the dorsal midline between the withers and tail. The heads of overo horses usually have extensive white markings. In contrast, the white body markings of tobiano horses cross the dorsal midline, and the white on the head is most often limited to stars, snips, and blazes. Horses can be registered as toveros (or tob-overos) when they have characteristics of both tobiano and overo. Additionally, the APHA registers horses of Paint, Quarter Horse, and Thoroughbred breeding without white patterning and Paint horses that are all white as breeding stock.[1]

Many coat color genes have been identified in lab animals that influence embryonic neural crest cells that are the precursors of melanocytes.[2,3] In mice, several genes control the location or amount of white in the coat,[2,3] and additional genetic loci modify the amount of white.[4] In Paint horses, different genes are thought to control overo and tobiano white patterning.[5] However, overo is a broad category of white patterning, with several subtypes including frame, calico, splashed white, and sabino.[5]

Occasionally, all-white foals are produced from Paint horse matings.[6,a] A small number of these horses have normal gastrointestinal function and

NOTES

are registered as breeding stock white, but most are affected by myenteric aganglionosis and fatal functional intestinal obstruction.[7] This condition is called overo lethal white syndrome (OLWS). Conditions similar to OLWS occur in mice, rats, and humans (Hirschprung's disease).[8–10] All rodent conditions and some forms of Hirschprung's in humans result from mutations in the endothelin receptor B (EDNRB) or one of its ligands, endothelin 3.[8–10] The endothelin-signaling pathway is essential for the development and migration of neural crest cells that ultimately form melanocytes and enteric neurons.[2,11] It is now known that substitution of lysine for isoleucine at residue 118 of EDNRB is responsible for OLWS in Paint horses,[12] and this mutation has been associated with the parental frame overo phenotype.[13] Most solid-colored horses are homozygous for the Ile118 allele of EDNRB (wild type), all parents of OLWS foals are heterozygous, and all OLWS foals are homozygous for the Lys118 allele.[12–14]

It has been suggested that all overo horses carry a copy of the Lys118 allele.[14] If true, 25% of overo breedings would result in the birth of an OLWS affected foal. Paint horse breeders report that the incidence of OLWS from overo breedings is much lower, and that some overo stallions never produce foals affected with OLWS. In a small breeding trial conducted in 1978, 6 of 76 (7.9%) overo breedings resulted in the birth of a foal affected with OLWS.[a] One explanation for the lower than 25% incidence of foals with OLWS is that not all overo horses carry the Lys118 allele, suggesting that overo white patterning is the result of more than one gene. We report here the EDNRB genotype and white patterning phenotype of 1000 horses to determine the incidence of the Ile118Lys EDNRB and describe its association with types of white patterning.[15]

2. Materials and Methods

Horses

Genomic DNA was obtained from horses admitted to the University of Minnesota Veterinary Teaching hospital for treatment, from client samples submitted for detection of the Lys118 allele, and from breeders. The vast majority (>90%) of samples were obtained from registered American Paint horses. A few samples were obtained from Pintos (white-patterned horses other than American Paint Horse, Quarter Horse, or Thoroughbred), Thoroughbreds, Mustangs, and American Miniature Horses. Paint Horse breeding farms tested included those that had a high incidence of births of foals with OLWS and those that had no OLWS. The solid-colored horses tested were of Quarter Horse and Thoroughbred breeding.

Genotyping

Genomic DNA was obtained from blood[b] or hair root bulbs (3 roots in 20 μl of 200 mM NaOH for 15 minutes at 70°C, followed by neutralization with 20 μl of 200 mM HCl and 20 μl of 100 mM Tris, pH 8.5). The EDNRB alleles were detected by use of allele-specific PCR, as described,[12] allowing each horse to be genotyped as homozygous for Lys118 or Ile118, or as heterozygous.

Phenotyping[1,5,15]

Horses were phenotyped by 2 authors (PDV, EMS) who observed the live horse or pictures taken of both sides of the horse including facial markings. Horses without white patterning that did not descend from white-patterned lines were designated as solid. Horses of white-patterned bloodlines were first classified phenotypically by use of the American Paint Horse color classifications of tobiano, overo, tovero, or breeding stock. Overo horses were further classified into 6 subtypes, and breeding stock horses were classified as solid breeding stock or white breeding stock.

Tobiano

White markings on the head are similar to solid-colored horses (Fig. 1). Typically, all 4 limbs are white. Body markings are distinct and regular, often with round or oval patterns. Flanks are dark and the tail is often of two colors. White patches are often vertically oriented, cross the dorsal midline, and can appear draped over shoulders.

Overo

The head is often extensively marked with white (Fig. 2). Typically, at least 1 limb is dark. White markings vary from distinct crisp white patches on the body to large irregular or roan areas. White coloration does not cross the back between the withers and tail. The tail is one color.

Tovero

These horses have tobiano and overo characteristics. The overo characteristic is most commonly apparent

Fig. 1. A tobiano.

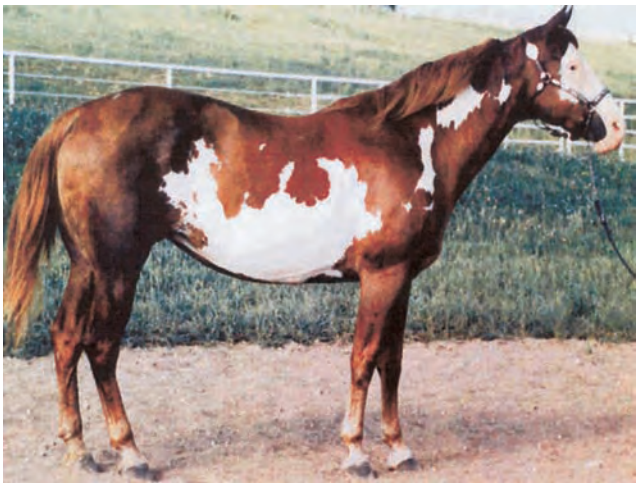

Fig. 2. A frame overo.

Fig. 3. A loud calico overo.

on the head, but can also be expressed by irregular body markings.

Breeding Stock

These are solid-colored horses of Paint lineage, including American Miniature Horses. Horses were subtyped as breeding stock white if they were adult all-white horses of Paint breeding.

Subtypes of Overo

Horses that fit the general description of overo have great variation in color pattern that allows further division into 6 distinct phenotypic subtypes: frame, calico, splashed white, sabino, medicine hat, and bald-faced.

Frame

A frame is an overo horse with body spots on the lateral aspect of the body (neck, shoulder, abdomen, flank, and hip) (Fig. 2). Body spots are distinct, with sharp borders, and do not connect with limb markings. At least 1 limb (often more) is dark. White limb markings do not usually extend up the carpus or tarsus. The head is usually extensively marked with white, which often extends laterally to the eyes. White spots that are primarily on the ventral midline or only involve the axilla are not typical of frame horses, especially when associated with extensively marked white legs.

Calico

A calico is an overo horse with white markings that are scattered and have irregular borders (Fig. 3). One or more limbs are white, and the white limb markings have narrow extensions up the limb. White markings on the limbs often connect to white markings on the body. Head markings are irregular and may be wide, but do not usually involve the eyes. Calico horses have the widest variation in the amount of white patterning. White-patterned horses without white patterning on the body but with irregular white limb markings extending above the tarsus and carpus were considered minimally marked calico overos. Horses with irregular limb markings above the tarsus and carpus and irregular white body patches on $<75\%$ of their body were considered calico overos, and horses with irregular limb markings above the tarsus and carpus and irregular white markings on $>75\%$ of their body were considered loud calico overos.

Fig. 4. A splashed white overo.

Splashed White

A splashed white horse is an overo horse with extensive white markings on the head and large, crisp white patches on the ventral aspect of the neck and thorax that often connect with extensive white patches on the forelimbs (Fig. 4). The hind limbs also have white markings that extend dorsally above the tarsus, and the ventral aspect of the abdomen is

Fig. 5. A sabino overo.

Fig. 6. A medicine hat overo.

white. A splashed white horse is often described as a horse dipped in white.

Sabino

A sabino is an overo horse with one or more white limbs and white markings on the face (Fig. 5). An extensive area of roan coloring (mixing of white hairs within colored patches) is the major characteristic of sabinos. Colored areas are also irregularly shaped and flecked with white that blends with small white patches.

Bald-Face

These are overo horses with white face markings that extend ventral to a line that connects the point of the mouth and the poll. The body coloring is solid, and white limb markings distal to the tarsus and carpus may be present.

Medicine Hat

This is a rare color pattern in which the coat is almost entirely white (Fig. 6). Pigmented areas are found primarily on the ears and poll, but may also appear on the thorax, flank, dorsal midline, and tail head. Medicine hat horses can arise from overo or tovero bloodlines; when of overo bloodlines, medicine hat horses may have pigment that is quite faint on the dorsal midline.

Overo Blends

Overo blends are those overo horses that have evidence of two or more overo white patterns (Fig. 7). There are two recognizable subdivisions of overo blends. The most common is the frame blend, which has frame characteristics such as lateral body spots, solidly colored limbs, and wide blazes, in addition to patterning of another overo subtype, which is usually calico. Less common are the other (nonframe) overo blends, which have two other types of overo patterning.

Fig. 7. A frame blend overo.

OLWS

Foals affected with OLWS are almost entirely white (Fig. 8). In most foals with OLWS, pigmentation is restricted to the retina. However, small pigmented areas may be present, usually in the mane or tail.

Nonpaint

These are registered Thoroughbreds and Quarter Horses that do not have excessive white on their faces or white limb markings proximal to the carpus or tarsus.

Statistics

Data were summarized by use of two-way tables. Horses were categorized by pattern versus 3 genotypes (homozygous Ile118, homozygous Lys118, and heterozygous). Chi-square analysis was used to test for association between pattern and genotype. Subtables were analyzed by use of chi-squared anal-

Fig. 8. A frame overo mare and her overo lethal white foal.

ysis or the Fisher exact test. Significance was established as $p < 0.05$.

3. Results

All foals with OLWS were homozygous for the Ile118Lys EDNRB mutation, and adults that were homozygous were not found. White patterning was strongly associated with EDNRB genotype ($p < 0.001$). Color patterns with highest incidence (>94%) of heterozygotes (carriers) were frame overo, highly white calico overo, and frame blend overo. White-patterned bloodlines with lowest incidence of carriers (<21%) were tobiano, sabino, minimally white calico overo, splashed white overo, nonframe blend overo, and breeding stock solid. The mutation was not detected in solid-colored horses from breeds without white patterning (Table 1). Looked at from another perspective, the white patterns appeared to segregate into three groups based on their incidence of carriers (Fig. 9). In the group where the gene was very common, it appears to be essential for the production of that pattern. In those color patterns with a middle incidence of carriers, the gene was non-essential and did not interfere with the production of that pattern, and in color patterns where the incidence was low, the gene is not essential and may be deleterious to the production of that phenotype.

4. Discussion

Of all frame and frame-blend overos tested in the study reported here, 96% possessed the Lys118 allele, strongly supporting the conclusion that in the heterozygous state, the Ile118Lys EDNRB mutation commonly causes the frame phenotype. However, calico overos with a large amount of white coloring had 100% incidence of the Lys118 allele, indicating that these largely white calico horses are actually frame blends. The frame patterning is unapparent because it magnifies the calico white pattern. Additionally, some heterozygote horses for the Lys118 allele do not express the frame overo pattern. Causes for non-frame phenotype heterozygotes include fusion with other white patterns such as tobianos (10% heterozygotes), splashed white overos (12%), calico overos (55%), and sabino overos (20%). Another cause is variable expression of the frame phenotype (bald-faced and breeding stock solid), possibly because of the influence of other genes.[16] Breeding stock horses of American Paint Horse lineage that carry the Lys118 allele always have some white markings, but 2 heterozygote American Miniature horses had no white markings, suggesting that there is a modifier gene present in the American Miniature horse population that strongly suppresses white patterning.

In addition to the nonframe heterozygotes, there are a few horses of apparent frame phenotype (5% of frame overos) that do not carry the Lys118 allele. The most common marking on these horses is a single distinct white patch on the ventral aspect of the abdomen that extends laterally up the frame. Variable expression of other white patterning genes is most likely the cause for these patterns. For example, splashed white horses have white body patches with sharp borders, and minimal expression of this pattern could look like frame patterning.

There appears to be 4 distinct overo patterns: frame, calico, splashed white, and sabino, and these subtypes of overo are under the control of different, independently assorting genes.[5] Our data support that hypothesis. The Lys118 allele causes the frame pattern, and splashed white, calico, and

Table 1. Distribution of Genotypes for Endothelin Receptor B in 945 White-Patterned Horses and 55 Solid-Colored Horses

Color Pattern	Number of Horses	N/N	N/L (%)	L/L
Overos				
Frame	188	10	178 (95)	0
Calico Loud	37	0	37 (100)	0
Calico	38	17	21 (55)	0
Minimal	67	61	6 (9)	0
Splashed White	26	23	3 (12)	0
Sabino	15	12	3 (20)	0
Bald faced	17	11	6 (35)	0
Medicine Hat	13	4	9 (69)	0
Overo Blends				
Frame blend	158	5	153 (97)	0
Non-frame blend	14	14	0 (0)	0
OLWS foals	28	0	0 (0)	28
Tobiano	109	98	11 (10)	0
Tovero	84	35	49 (58)	0
Breeding stock				
Solids	146	120	26 (18)	0
All-white	5	1	4 (80)	0
Solid-colored horses	55	55	0 (0)	0

N/N = wild type genotype; N/L = heterozygote; L/L = homozygous for OLWS mutation.

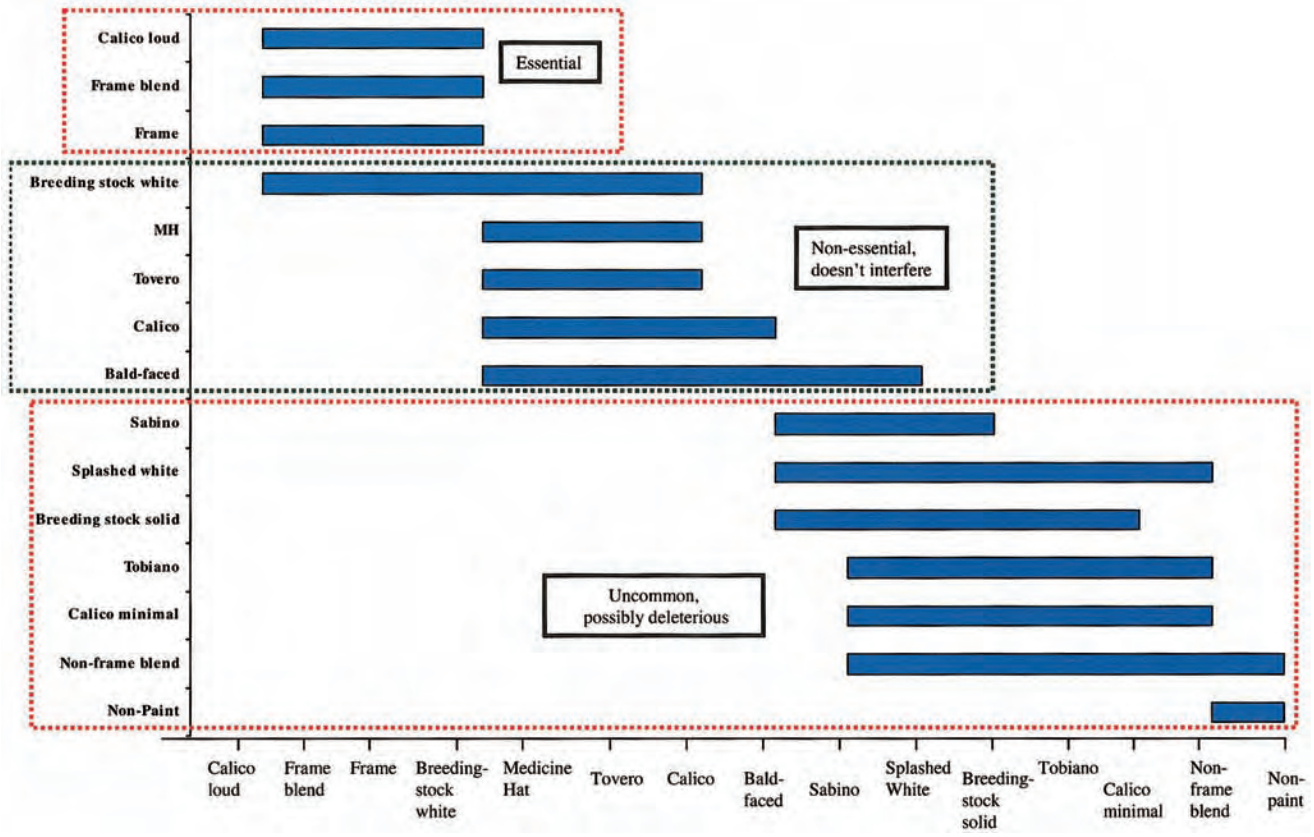

Fig. 9. EDNRB genotype associations among horses tested. White patterns on the *x*-axis have a similar incidence of heterozygotes as white patterns on the *y*-axis where they share a color bar.

sabino horses have a significantly different incidence of the Lys118 allele, indicating a distinct genetic basis. Additionally, these 4 overo patterns commonly appear blended, as would be expected from independently segregating overo genes. When combined with other overo white patterns, the Lys118 allele appears to add more white on the head and frame of the body. This is best seen in calico overos in which the percentage of heterozygotes increases from 9% in minimally white calicos, to 55% in moderately white individuals, to 100% in the whitest calicos.

Tobiano is known to have a genetic basis distinct from overo, and there is low incidence (10%) of the Lys118 allele in these horses. However, if breeders choose a tobiano mate for a horse that is heterozygous for Lys118 to avoid OLWS, they will not always achieve their goal. Our experience is that tobiano horses are only heterozygous for the Lys118 allele when their pedigree contains overo horses. The 18% incidence of heterozygotes in breeding stock solid horses again suggests that the frame phenotype either has variable expression or may be suppressed by other genes. Nonframe blends are horses that have two overo patterns other than frame, most commonly sabino and calico. Their existence and lack of heterozygotes among them supports the hypothesis that overo horses are genetically heterogeneous.

Additional support for genetic heterogeneity of overo patterning can also be inferred from the <25% incidence of OLWS foals from overo breeding.[a] In the study reported here, 73% percent of all overo and overo blend horses were heterozygous for the Lys118 allele. If our samples were representative of overos in general, the predicted incidence of births of foals affected by OLWS in overo × overo breeding would be 13.3% (i.e., 0.73 × 0.73 × 25%). This figure agrees with anecdotal reports from breeders of Paint horses, but is slightly higher than the figure of 7.9% detected in a small breeding trial.[a] Our finding that 27% of overo horses did not possess the Lys118 allele explains why some overo breedings never produce foals affected by OLWS.

A few white-patterned horses of breeds other than American Paint horses (Thoroughbreds, American Miniature Horses, and half Arabians) were heterozygous for the Lys118 allele. This may be deliberate, because breeders outcross to American Paint Horses to add white patterning. When heterozygotes are used for outcrossing, this gene will be introduced into the breed, along with the possibility of producing foals affected by OLWS. However, the appearance of the Lys118 allele in breeds unrelated

to American Paint Horses is more difficult to explain. Undocumented outcrosses to white-patterned horses are a possibility. De novo mutations have also been suggested to be responsible for the birth of white-patterned horses from solid parents,[13,16] but this is yet unproven. We believe that these horses may also result from white patterning genes in solid breeds that are minimally expressed because of variable expression or suppression by other genes. If this is true, the Lys118 allele entered these breeds many years ago.[13]

Of greater importance, at least to some breeders, is the occurrence of white patterning in Quarter Horses. Foals with excessive white markings born to registered Quarter Horse parents are known as crop-outs, and are ineligible for registration by the American Quarter Horse Association. It is known[13] or suggested by breeding results[a] that at least some crop-out Quarter Horses are heterozygous for the Lys118 allele. This study tested 25 Quarter Horses that were either crop-outs or parents of a white-patterned horse, and did not find any heterozygotes, lending further support to the concept that other genes control overo coloration. As genetic testing becomes available for more overo genes, testing of crop-outs and their parents will help determine whether the crop-out white patterning is the result of de novo mutations or whether white patterning genes exist in solid-colored Quarter Horses.

5. Conclusion

The Ile118Lys EDNRB mutation causes OLWS when a foal inherits a copy from each parent. Carriers of the mutation most commonly exhibit a frame overo phenotype, but the frame pattern can be suppressed or combined with other white patterns, making accurate estimation of EDNRB genotype by visual inspection difficult. Determination of EDNRB genotype by use of a DNA test is the only way to determine with certainty whether white-patterned horses can produce a foal affected with OLWS. If carrier-to-carrier matings are prevented, breeders can eliminate the production of lethal white foals.

Supported by the Minnesota Agricultural Experiment Station.

The authors thank Judith L. Morris, L. A. Feichtinger, A. Purdy, and Drs. J. Hurtgen, J. S. Juzwiak, M. Ketchum, C. Landa, N. McEachern, and S. J. Valberg for technical assistance, and Drs. Murray K. Clayton and Ryland B. Edwards for statistical analysis.

References and Footnotes

1. American Paint Horse Association Official Rule Book, 33rd edition Fort Worth, TX: American Paint Horse Association, 1998;232–233.
2. Barsh GS. The genetics of pigmentation: From fancy genes to complex traits. *Trends Genet* 1996;12:299–305.
3. Jackson IJ. Molecular and developmental genetics of mouse coat color. *Annu Rev Genet* 1994;28:189–217.
4. Pavan WJ, Mac S, Cheng M, et al. Quantitative trait loci that modify the severity of spotting in piebald mice. *Gen Res* 1995;5:29–41.
5. Sponenberg DP. Patterns of white. In: *Equine color genetics.* Ames: Iowa State University Press, 1996;53–82.
6. Trommershausen-Smith A. Lethal white foals in matings of overo spotted horses. *Theriogenology* 1977;8:303–311.
7. Hultgren BD. Ileocolonic aganglionosis in white progeny of overo spotted horses. *J Am Vet Med Assoc* 1982;180:289–292.
8. Hosoda K, Hammer RE, Richardson JA, et al. Targeted and natural (piebald-lethal) mutations of endothelin-B receptor gene produce megacolon associated with spotted coat color in mice. *Cell* 1994;79:1267–1276.
9. Gariepy CE, Cass DT, Yanagisawa M. Null mutation of endothelin receptor type B gene in spotting lethal rats causes aganglionic megacolon and white coat color. *Proc Natl Acad Sci U.S.A.* 1996;93:867–872.
10. Puffenberger EG, Hosoda K, Washington SS, et al. A missense mutation of the endothelin-B receptor gene in multigenic Hirschsprung's disease. *Cell* 1994;79:1257–1266.
11. Baynash AG, Hosoda K, Giaid A, et al. Interaction of endothelin-3 with endothelin-B receptor is essential for development of epidermal melanocytes and enteric neurons. *Cell* 1994;70:1277–1285.
12. Santschi EM, Purdy AK, Valberg SJ, et al. Endothelin receptor B mutation associated with lethal white syndrome in horses. *Mam Gen* 1998;9:306–309.
13. Metallinos DL, Bowling AT, Rine J. A missense mutation in the endothelin-B receptor gene is associated with Lethal White Foal Syndrome: an equine version of Hirschprung's disease. *Mam Gen* 1998;9:426–431.
14. Yang GC, Croaker D, Zhang AL, et al. A dinucleotide mutation in the endothelin-B receptor gene is associated with lethal white foal syndrome (LWFS); a horse variant of Hirschsprung disease (HSCR). *Hum Mol Gen* 1998;7:1047–1052.
15. Santschi EM, Vrotsos PD, Purdy A, Mickelson JR. Incidence of the endothelin receptor B mutation that causes lethal white foal syndrome in white patterned horses. *Am J Vet Res* 2001;62:97–103.
16. McCabe L, Griffin LD, Kizner A, et al. Overo Lethal White Foal Syndrome: equine model of aganglionic megacolon (Hirschprung's disease). *Am J Med Gen* 1990;36:336–340.

[a]Metzger IL. The overo white cross in spotted horses [thesis]. Columbia, MO: University of Missouri; 1978.
[b]Puregene, Gentra Systems Inc, Research Triangle Park, NC.