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Evaluation of deafness in American Paint Horses by phenotype, brainstem auditory-evoked responses, and endothelin receptor B genotype

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EQUINE

Objective—To evaluate deafness in American Paint Horses by phenotype, clinical findings, brainstem auditory-evoked responses (BAERs), and endothelin B receptor (EDNBR) genotype.

Design—Case series and case-control studies.

Animals—14 deaf American Paint Horses, 20 suspected-deaf American Paint Horses, and 13 nondeaf American Paint Horses and Pintos.

Procedures—Horses were categorized on the basis of coat color pattern and eye color. Testing for the EDNBR gene mutation (associated with overo lethal white foal syndrome) and BAERs was performed. Additional clinical findings were obtained from medical records.

Results—All 14 deaf horses had loss of all BAER waveforms consistent with complete deafness. Most horses had the splashed white or splashed white–frame blend coat pattern. Other patterns included frame overo and tovero. All of the deaf horses had extensive head and limb white markings, although the amount of white on the neck and trunk varied widely. All horses had at least 1 partially heterochromic iris, and most had 2 blue eyes. Ninety-one percent (31/34) of deaf and suspected-deaf horses had the EDNBR gene mutation. Deaf and suspected-deaf horses were used successfully for various performance events. All nondeaf horses had unremarkable BAER results.

Conclusions and Clinical Relevance—Veterinarians should be aware of deafness among American Paint Horses, particularly those with a splashed white or frame overo coat color pattern, blend of these patterns, or tovero pattern. Horses with extensive head and limb markings and those with blue eyes appeared to be at particular risk. (*J Am Vet Med Assoc* 2009;235:1204–1211)

Deafness is infrequently recognized in horses. Reported causes of deafness include trauma, inflammation or infection of the peripheral auditory pathways (eg, temporohyoid osteoarthropathy or otitis interna or media), and suspected gentamicin intoxication.[1–3] Reported causes of deafness in other species that might have clinical relevance to horses include ototoxic agents such as loop diuretics, presbycusis (age-related hearing loss), acoustic-nerve tumors, and congenital sensorineural deafness.[4] In several dog and cat breeds as well as in laboratory animal species and humans, sensorineural deafness is often associated with pigmentation alterations of the skin, irides, or both.[5–7]

Lack of functional melanocytes is the link between deafness and pigment alterations.[8] Hearing and pigmentation of the skin are similar in that a small population of melanocytes within the inner ear is essential for hearing.[9] Although the specific role of melanocytes in the ear is unknown, the cells are important for cochlear function.[8] Melanocytes are derived from the neural crest and are contained within a blood-vessel rich zone of the cochlea known as the stria vascularis.[8,9] The stria plays a role in modulating the chemical composition of endolymph, particularly potassium concentration.[9] Without melanocytes in the inner ear, the stria vascularis does not develop or function typically and may degenerate along with cochlear hair cells and auditory neurons.[8] Congenital deafness is associated with abnormal migration of these melanocytes from the neural crest and poor survival or development within the inner ears of some animals with coat and iris color pigmentation alterations (white spotting).[8,9]

Genetic mutations that cause spotted coat color as well as hearing impairment have been detected in several dog breeds, white cats, mink, and rodents as well as humans with Waardenburg syndrome.[8] Whether an association between coat color and hearing impairment exists in horses is unknown. Furthermore, the phenotype of deaf horses has not yet been defined. Because

Abbreviations

APHA	American Paint Horse Association
BAER	Brainstem auditory-evoked responses
EDNBR	Endothelin B receptor
OLWFS	Overo lethal white foal syndrome

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American Paint Horses have white spotting and commonly have blue irides, such an association is plausible. Deafness in an overo Paint gelding has been reported.[10] The purpose of the study reported here was to investigate whether alterations in coat color patterns were associated with deafness in horses. Specifically, we sought to evaluate deafness in American Paint Horses by coat pattern phenotype, clinical findings, BAERs, and EDNBR genotype.

Materials and Methods

Horses—Four groups of horses were used in the study. The first group (confirmed-deaf horses) consisted of American Paint Horses that were evaluated for deafness or suspected OLWFS at the William R. Pritchard Veterinary Medical Teaching Hospital, University of California-Davis, from 1990 to 2007. All confirmed-deaf horses were reportedly determined to be deaf at or shortly after birth or from the time of acquisition by the current owner. None had any known cranial trauma, illness, gestational problem, or exposure to ototoxic agents that could account for deafness. The second group (suspected-deaf horses) consisted of American Paint Horses with owner-suspected deafness, and information about these horses was obtained with a questionnaire. Participants in this group were recruited via placement of a notice in the *American Paint Horse Journal*. These horses were not examined by the investigators. The third group (nondeaf horses to serve as control subjects for BAER and genotype tests) consisted of Paint or Pinto horses that were evaluated at the teaching hospital for problems other than deafness and for nonneurologic reasons (some horses were related to deaf horses and were brought along by the owners). These horses were hearing tested and responded unremarkably in terms of behavioral response to auditory stimuli, indicating they were not deaf. Consent for participation of confirmed-deaf and nondeaf horses was obtained from owners in accordance with hospital protocols for clinical studies. The fourth group consisted of horses identified in the hospital database (1985 through 2008) as deaf as diagnosed via BAER testing or as suspected deaf on the basis of results of clinical examinations. Breed was recorded for horses identified with idiopathic or congenital deafness.

Phenotypic analysis—Phenotypic analysis (coat color and pattern, iris color, and face and limb markings) was performed for the confirmed-deaf, suspected-deaf, and nondeaf horses. Clinical findings in confirmed-deaf horses (signalment, history, and results of diagnostic tests, physical examination, and neurologic examination) were also recorded.

For phenotypic analysis, horse coat patterns were classified as tobiano, overo, or tovero (**Appendix**).[11–14] Overo horses were further subclassified according to the 3 major genetically distinct overo patterns recognized by the APHA, including frame, splashed white overo, and sabino.[11–14] The classification of tovero, frame–splashed white overo blend, or frame-sabino overo blend was assigned to horses that had > 1 coat color pattern.[11,15] For 25 years, the person who classified the horses (KGM) has been involved in the evaluation of coat color pattern in American Paint Horses. Photographs of deaf horses' parents and grandparents were available through the APHA[16] and were used to aid in categorization of coat color patterns.

The most common overo pigmentation pattern is frame overo,[11] and the gene for frame overo patterning is semidominant. In the heterozygous state, the gene yields the frame overo phenotype. In the homozygous state, the gene is associated with OLWFS, which is associated with a mutation in the EDNBR gene.[13,15] The genetic mutation causing the most common form of sabino overo, including the form in APHA horses, has been described and named sabino-1.[14] The mode of inheritance of the sabino phenotype, like frame overo, appears to be semidominant, with heterozygotes commonly having 4 white limbs, ventral body spots with flecks, and white on the head, whereas homozygous horses have an all- or nearly all-white phenotype.[14] Although there are genetic tests available for frame overo and sabino-1, there is no genetic test for detecting a splashed white overo horse. Crosses of these coat color patterns are common among horses registered with the APHA. Tovero horses are those with both tobiano and overo patterns and are often mostly white. Combinations of overo patterns in horses also tend to result in more white than a horse with a single pattern would have. Examples would include frame–splashed white or frame-sabino overo blend.[15]

Clinical assessment—Radiography of the head, CBCs, serum biochemical analyses, serologic testing, and cytologic evaluation of the CSF were variably performed for the confirmed-deaf American Paint Horses. Otoscopic and neurologic examinations were also variably performed. Long-term follow-up information on confirmed-deaf horses was obtained through telephone calls to owners.

BAER testing—Brainstem auditory-evoked responses were evaluated in confirmed-deaf and nondeaf horses as described elsewhere.[2] Briefly, 1 of 2 evoked-potential recording systems[a,b] was used. Horses were placed in examination-room stocks and sedated with xylazine (0.2 to 0.6 mg/kg [0.09 to 0.27 mg/lb], IV) or detomidine hydrochloride (7 to 12 µg/kg [3.2 to 5.4 µg/lb], IV) and butorphanol tartrate (8 to 12 µg/kg [3.6 to 5.4 µg/lb], IV). One horse was tested while anesthetized with halothane for concurrent myelography. Platinum-alloy needle electrodes were inserted SC in each horse by use of a hospital protocol for BAER in horses.[2] One electrode was placed at the vertex on the midline at the center point between the intercanthus and the occipital protuberances. Left and right mastoid electrodes were positioned ventral to the most caudal extent of the zygomatic arch. A recording electrode was placed on the dorsal midline at the level of C2. A ground electrode was placed between the occipital protuberance and C2. Simultaneous recordings were obtained with 2 electrode combinations/tested ear: vertex to ipsilateral mastoid process and vertex to C2.

Genotyping—Genotyping was performed for confirmed-deaf, suspected-deaf, and nondeaf horses. Genetic analysis for the EDNBR gene mutation associated

with OLWFS was performed as described elsewhere.[13] In brief, the EDNBR gene was sequenced and evaluated for a 2-base pair change in the first exon at base pairs 353 and 354 of the cDNA.[13] This nucleotide change corresponds to an amino acid substitution of lysine for isoleucine at position 118.[13]

Statistical analysis—Descriptive statistics were calculated to summarize signalment of the confirmed-deaf horses. Results are reported as mean ± SD. The Fisher exact test was used to determine whether there was an association between deafness and heterozygosity of the EDNBR gene mutation in both confirmed- and suspected-deaf horses, and odds ratios and 95% confidence intervals were calculated. Statistical significance was set at values of $P \leq 0.05$.

Results

Horses—Eleven deaf American Paint Horses as well as 3 neonatal white American Paint Horse foals that were evaluated for suspected OLWFS at 12 to 24 hours after birth were assigned to the confirmed-deaf group. Among the 11 deaf American Paint Horses, there were 7 fillies or mares, 2 geldings, and 2 stallions. Ages ranged from 1 to 6 years, with a mean ± SD age of 2.6 ± 1.5 years. Thirteen American Paint Horses or Pintos with white markings were used as nondeaf control horses for BAER and genotype testing.

Nineteen owners with a total of 20 horses (11 female and 9 male) responded to the study advertisement and provided horse photographs, horse pedigrees, horse hair for DNA analysis for the EDNBR gene mutation, and answers to questions regarding horse use and their own perceptions of limitations or unusual behavior associated with the deafness. Most horses were being used in performance events despite the suspected deafness. Eight horses were used for pleasure or trail riding, 5 were used for breeding purposes, 5 were used for halter showing, 5 were too young for riding but were intended for training, 2 were used for western pleasure showing, and 2 were described as show horses (several horses served > 1 purpose). Additional uses included dressage, team penning, jumping, pulling carts, working cow horse competition, and lunge-line competition.

Phenotypic analysis—Base coat colors of confirmed-deaf horses included chestnut or sorrel (n = 6), bay (4), and palomino (1). Coat patterns included overo (10) and tovero (tobiano-overo cross; 1; **Table 1**). Frame–splashed white overo blend and splashed white overo patterns were most common (**Figures 1 and 2**). The amount of white spotting varied widely, from > 50% of the coat surface area to minimal white. All 11 horses had 1 or more blue irides and, commonly, at least partially nonpigmented palpebral skin (**Figure 3**). Eight horses had bilateral, completely blue irides. Two horses had a unilateral, completely blue iris with a brown contralateral iris; 1 horse had 1 completely blue iris and 1 partially heterochromic iris. All 11 horses had extensive white facial markings; 4 horses had bald faces and 7 had apron to bonnet faces. All also had 2 or more white limb markings; 7 horses had 4 white limbs, 3 had 3 white limbs, and 1 had 2 white limbs.

The 3 foals with suspected OLWFS had white coat color on > 98% of their body and bilateral blue irides. One foal was the progeny of 2 frame overo horses, the second originated from the mating of an overo with a tovero horse, and the third was the result of a mating of an overo horse with a breeding-stock Paint horse. The breeding stock (solid-colored) stallion was sired by a frame overo horse, allowing for the possibility of inheriting the EDNBR gene mutation associated with OLWFS. Owners were unaware of any hearing impairments in any parent.

Among the 20 suspected-deaf horses, the 2 most common phenotypes were similar to those of the confirmed-deaf horses: splashed white overo and frame–splashed white overo blends were most common (Table 1). There was 1 white horse, which originated from the mating of a frame overo sire and completely white dam. The dam had been the product of an overo to overo horse mating. All horses had extensive white markings on the limbs; 17 (85%) had white markings on all 4 limbs, 2 (10%) had 2 or 3 white limbs, and it was impossible to delineate limb markings from the photographs provided for 1 horse. All 20 horses had

Table 1—Number of confirmed-deaf (n = 14), suspected-deaf (20), and nondeaf (13) horses with specific coat patterns typical of American Paint Horses.

Group	Tobiano	Frame overo	Splashed white overo	Sabino overo	Tovero	All white	Overo blends	Other
Confirmed-deaf horses	0	1	4	0	1 (tobiano with splashed white)	3 (all lethal white)	5 frame–splashed white	NA
Suspected-deaf horses	0	2	6	0	2	1 (nonlethal white; suspect overo blend)	6 frame–splashed white and 3 frame-sabino	NA
Nondeaf horses	2	0	0	1	4	1 (nonlethal white; sabino overo homozygote)	1 frame–splashed white and 1 frame-sabino	1 breeding stock (minimal white; splashed white) and 2 minimal white (possible splashed white or sabino)

NA = Not applicable.
See Appendix for descriptions of coat patterns.

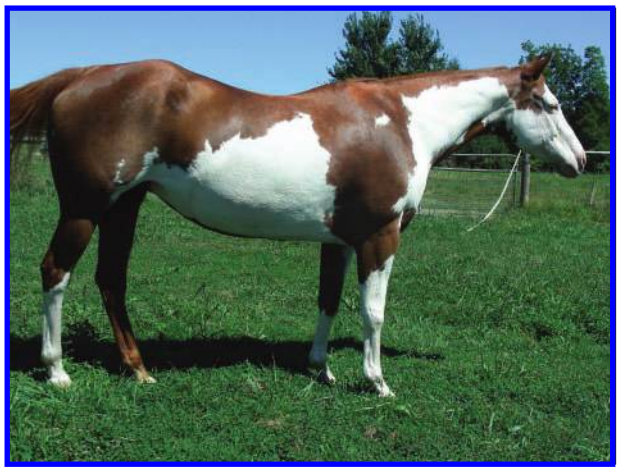

Figure 1—Photograph of a deaf horse with frame–splashed white overo blend white patterning.

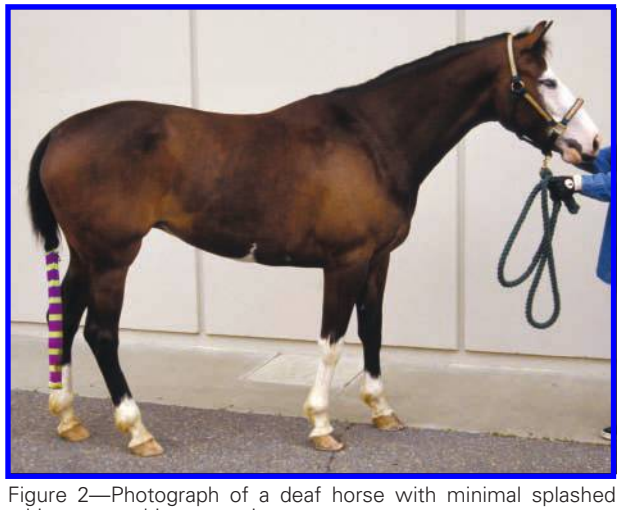

Figure 2—Photograph of a deaf horse with minimal splashed white overo white patterning.

Figure 3—Close-up photograph of a deaf horse with typical eye and facial markings of deaf horses.

extensive white on the face (apron to bonnet face or completely white) and at least 1 partially heterochromic eye. Most (15 [75%]) had 2 blue eyes, 1 horse had 1 blue and 1 partially blue eye, 1 horse had 1 blue and 1 brown eye, and 2 horses had 1 partially heterochromic and 1 brown eye.

Nine of the 13 nondeaf horses were American Paint Horses, with variable coat patterns (Table 1). All of these 9 horses had 3 or 4 white limbs and bald, apron, or bonnet faces. Despite having been registered as a breeding stock Paint, 1 mare had 1 blue eye and a bald face and was believed to represent a minimally expressed splashed white overo on the basis of the phenotype of its parents and full siblings. An additional 3 Pintos were tested; these were nonregistered Pintos of mixed breeding, including 2 tobiano horses and 1 tovero horse, the latter of which had a bonnet face, 4 white limbs, and 2 blue eyes. The thirteenth horse, believed to be a homozygous sabino overo horse, was a white Tennessee Walking Horse with brown-pigmented irides and nonpigmented skin. Ten nondeaf horses were evaluated at the teaching hospital for problems other than hearing impairment. Three horses were related to confirmed-deaf horses used in the study and were evaluated for the purpose of serving as nondeaf control horses; 1 horse was a tovero dam of a deaf tovero gelding, a second was a breeding-stock (solid-colored) full sister to a deaf splashed white overo mare, and another was a yearling sabino overo progeny of a deaf splashed white overo stallion. All the overo or tovero nondeaf horses had extensive white markings on the head (bald to bonnet), and 4 had blue irides and 3 or more white limbs. The tovero horses were similar in phenotype to the confirmed-deaf tovero in the study. The frame–splashed white overo blend mare was similar in phenotype to several confirmed-deaf overo horses.

Clinical findings—Results of physical and neurologic examinations were unremarkable in 10 of 11 confirmed-deaf American Paint Horses except for a lack of behavioral responses to auditory stimuli that were produced outside of the visual field. One of the horses was quadrilaterally ataxic (grade 2/5) in addition to being clinically deaf. The ataxia had been detected 6 months prior to evaluation and was attributed to cervical vertebral compressive myelopathy at C3-4, as diagnosed via myelography. Results of otoscopic examination of both external auditory canals and examination of the ears were unremarkable in all horses. Results of CBCs and serum biochemical analyses were also unremarkable. Radiographs of the head revealed no abnormalities in all 7 horses that received them. In 2 horses, CSF had been collected and was cytologically unremarkable. These CSF samples were also free of antibodies against *Sarcocystis neurona* as revealed by western blot analysis. Follow-up with owners 3 months to 3 years after the initial evaluation (17 years afterward for 1 horse) revealed the 11 horses had remained clinically normal except for deafness.

The 3 foals with suspected OLWFS had colic signs of varying severity and abdominal distention. Two of these foals reportedly had poor affinity to their dams and lacked a behavioral response to mare vocalizations, even before development of colic. None had passed meconium despite the administration of enemas; all had become progressively distended, with persistent signs of colic, and had been anorectic since shortly after birth. Abdominal ultrasonography, survey abdominal radiographs, and radiographs with barium contrast en-

emas in 2 foals revealed findings consistent with ileus and reduced diameter of the small colon or rectum as well as distention of the small intestine. Administration of high-retention enemas resulted in the passage of white to yellow mucus and clear fluid with no fecal staining or material in all 3 foals. The foals were unable to defecate the rectally administered barium contrast material. Results of hematologic and serum biochemical analyses suggested hemoconcentration consistent with hypovolemia. The oldest foal, which had signs of stupor and hypovolemic shock, also had leukopenia with a shift to the left. Results of neurologic examination were unremarkable in 2 foals, with the exception of a lack of behavioral response to auditory stimuli; neurologic examination in the third foal was not possible because of its stuporous mentation. All 3 foals had unremarkable findings for otoscopic examination and external ear anatomy. Given the clinical status of the foals, their phenotype, their parentage, the results of diagnostic tests, the lack of any feces production, and progressive signs of abdominal discomfort, a presumptive diagnosis of intestinal aganglionosis (OLWFS) was made. The owners elected for euthanasia because of a poor prognosis. Intestinal aganglionosis consistent with OLWFS was confirmed in all foals at postmortem examination.

BAER testing—Recordings of BAERs confirmed bilateral deafness with complete lack of waveforms in 10 of 10 deaf American Paint Horses tested. Complete bilateral deafness was also confirmed in the 3 foals with OLWFS. All 13 nondeaf horses had results of BAER testing that would be expected of hearing horses.

Genotyping—Eleven confirmed-deaf horses (8 deaf American Paint Horses and 3 foals with OLWFS) were genotyped for the EDNBR mutation that causes OLWFS. Seven of the 8 confirmed-deaf American Paint Horses were heterozygous for the genetic mutation, whereas 1 horse did not have the mutation. Five of the 11 confirmed-deaf horses were directly related to one another, despite being from different farms and locations within California or Nevada, and were traced back to a single overo stallion. All 3 foals with OLWFS were homozygous for the EDNBR gene mutation. Overall, 3 of 7 genotyped nondeaf horses were heterozygous for the mutation.

The EDNBR gene mutation was detected in the heterozygous state in 18 of the suspected-deaf horses; 2 did not have the mutation. Two of the suspected-deaf horses were closely related to 2 of the confirmed-deaf horses; 1 suspected-deaf horse was sired by the same stallion as a confirmed-deaf horse. A second suspected-deaf horse was the progeny of the same mare as one of the confirmed-deaf horses, and its sire was the same stallion as 5 of the confirmed-deaf horses.

In total, 91% (31/34) of confirmed- and suspected-deaf horses had the EDNBR genetic mutation. Deaf horses (confirmed-deaf and suspected-deaf horses combined) were significantly (P = 0.013) more likely to have the EDNBR gene mutation than were nondeaf horses (odds ratio, 12.4; 95% confidence interval, 1.8 to 84.3).

Survey of owners of 20 suspected-deaf horses—Owner responses to questions regarding perceived limitations or problems associated with deafness revealed 2 horses were slightly more skittish than typical, 1 horse moved its ears more than usual, 1 with thickened ear margins appeared reluctant to have its head touched, 1 had floppy ears, 1 bit or nipped excessively, and 1 appeared nervous when housed in an enclosed stall. It is unknown whether these conditions were directly related to the deafness, but owner perceptions were that they may have been. Two owners believed their deaf horse was at increased risk of injury (kicking or biting) by other horses, and 2 others believed their horse was challenging to train because of the ineffectiveness of owner vocal commands. Some owners reported their horses appeared less shy or fearful than hearing horses, whereas others believed their horses were more easily startled by visual stimuli.

Breed association with deafness—The medical record search revealed 11 additional deaf or suspected-deaf horses examined at the teaching hospital. Eight of these horses had a diagnosis of temporohyoid osteoarthropathy, which has been associated with deafness.[2] Another horse had bilateral, severe otitis externa and interna. The only horses with no apparent cause for deafness included 2 Paint or Pinto horses (1 overo and 1 tovero) that were not included in the confirmed-deaf horse group. Both horses were judged clinically deaf by their owners and hospital clinicians; however, the deafness had not been confirmed with BAER testing.

Discussion

To the authors' knowledge, this is the first case series of horses with deafness associated with pigmentation alterations (white coat spotting or blue irides) in the peer-reviewed literature. It is also the first study in which the phenotype of deaf American Paint Horses was characterized and in which deafness was investigated in foals with OLWFS, providing more evidence that deafness may be associated with pigment mutations in horses. In addition, a medical records search revealed no other breeds of horses with idiopathic or congenital deafness. All deaf horses of other breeds had acquired causes of deafness, including temporohyoid osteoarthropathy and otitis. This adds further to the evidence of a breed-associated deafness in American Paint Horses.

Overo lethal white foal syndrome develops in foals homozygous for a mutation in the EDNBR gene, usually as a result of overo to overo horse matings, and leading to an all-white or nearly all-white coat, blue irides, and intestinal aganglionosis.[15] Sensorineural deafness associated with white coat spotting and blue eye color has been reported for humans,[9] cats,[4,5,17] dogs,[4] camelids,[18] and rodents[7–9] and is suspected in cattle.[19] Abnormal melanocyte migration or differentiation can result in several white-spotting and white coat color patterns that are often associated with blue irides.[8,9] In dogs, 2 main pigmentation genes, merle and piebald, are associated with congenital sensorineural deafness.[20,21] Cats with white pigmentation and blue irides are often deaf, and heredity of this group of characteristics is autosomal dominant.[5]

With the exception of the 3 foals with OLWFS and 1 adult frame overo horse, all confirmed-deaf horses in the present study had a splashed white overo coat pattern or a frame–splashed white overo blend. In ad-

dition, more than half (60%) of suspected-deaf horses identified via questionnaire had a splashed white or frame–splashed white overo blend. This suggested that the splashed white overo pattern may be associated with deafness more than other overo patterns, particularly in light of the fact that splashed white is reportedly one of the least common overo patterns.[11,15] It should be considered that some of the splashed white horses in the present study were minimal overos (ie, they only had a bald face, blue eyes, and white pigmentation below the carpi and tarsi in 3 or 4 limbs). Given the results of our study, the amount of white spotting associated with deafness in horses is highly variable. However, white spotting on the face and limbs and blue coloration of irides appeared to be consistent features in deaf horses because all confirmed-deaf and suspected-deaf horses in this study had bald, apron, or bonnet faces; 1 or 2 blue or partially heterochromic eyes; and 2 or more white limbs. The frame overo pattern, which is the most common overo pattern among horses registered with the APHA, also appeared to be associated with deafness because one of the study horses and the 3 foals with OLWFS had a frame overo pattern (OLWFS being maximal or homozygous expression of the phenotype). In addition, 10% of the suspected-deaf horses had a frame overo pattern and 45% had a frame pattern blended with other overo patterns. None of the confirmed-deaf horses examined at our hospital nor any of the suspected-deaf horses had the tobiano or sabino overo phenotype. Given that approximately 36% of APHA-registered horses are reportedly tobiano, 24% are overo, and 5% are tovero,[a] tobiano horses were underrepresented among confirmed- or suspected-deaf horses in our study.

All of the confirmed- and suspected-deaf horses in our study had 1 or more blue or partial heterochromic iris. In cats, the likelihood of deafness increases with the number of blue eyes.[22] In Dalmatians, English Setters, and English Cocker Spaniels, the presence of blue irides also increases the likelihood of deafness.[6] Furthermore, Border Collies with blue irides and more white on the head than is typical of the breed have a higher prevalence of deafness.[20] Like deaf Border Collies, all of the suspected- and confirmed-deaf horses in the present study also had large amounts of white on the face.

In Dalmatians, unilateral deafness is far more prevalent than bilateral deafness.[23] It is unknown whether this is the situation in horses because it would be difficult to identify unilaterally deaf horses without BAER testing. However, identification of such horses is clinically important in terms of breeding because of the possibility that deafness would be inherited by offspring. In puppies, having 1 or more deaf (even unilaterally deaf) parents is positively associated with deafness in several spotted breeds,[6] and the odds of deafness in Border Collie puppies increases by a factor of 14 when their dam is deaf.[20]

Humans can be affected with several syndromes in which hypopigmentation and deafness coexist. Waardenburg syndrome is particularly relevant to this discussion in the parallels that can be drawn with the confirmed- and suspected-deaf horses of the present study. Waardenburg syndrome is the most common condition involving pigmentary anomalies and sensorineural deafness that result from the absence of melanocytes in the skin and the stria vascularis of the cochlea.[24] The syndrome is clinically and genetically heterogeneous. As with overo horses, the hypopigmentation in Waardenburg syndrome is patchy and includes the skin, hair, and irides.

Four subtypes of Waardenburg syndrome exist. Type I includes hypopigmentation and deafness as well as craniofacial defects, type II may be limited to hypopigmentation and deafness and is similar to type I except that it lacks dystopia canthorum, type III includes musculoskeletal deformities in addition to hypopigmentation and deafness, and type IV (Waardenburg-Shah syndrome) includes deafness and hypopigmentation in conjunction with Hirschsprung's disease.[9,25] Hirschsprung's disease is analogous to OLWFS in that it is characterized by megacolon caused by a deficient number of neural crest–derived enteric ganglia and is associated with a mutation in the EDNBR gene.[13] Type II Waardenburg syndrome involves a defect in hearing and pigmentation that may exist without concurrent obvious defects.[9,25] Therefore, types II and IV appear to be similar to characteristics of the confirmed- and suspected-deaf American Paint Horses of the present report. Certainly, clinical characteristics of the deaf foals with OLWFS and the deaf frame overo horses could be compared with those of humans with type IV Waardenburg syndrome. Type II Waardenburg syndrome is caused by a mutation in the *Mitf* gene, which is a central mediator of melanocytes development.[9] Type IV Waardenburg syndrome is associated with a mutation of the *SOX10*, endothelin-3, or EDNBR genes.[9] All of these genes play a role in neural crest development and modulate *Mitf* gene expression; each should be explored for a potential association with deafness in overo horses.

Most confirmed- and suspected-deaf horses in the present study were heterozygous for the EDNBR gene mutation associated with OLWFS, and the 3 white neonatal foals were homozygous for this mutation. In contrast, another study[26] revealed that only 21% of randomly selected APHA-registered horses were heterozygous for the mutation. Deaf horses (confirmed- and suspected-deaf horses combined) in our study were more likely to be carrying the EDNBR gene mutation than were nondeaf horses. However, this finding should be interpreted with caution because of small sample sizes and the fact that the nondeaf horse group had relatively fewer splash and frame overo horses. The finding does not explain the combination of hypopigmentation, blue irides, and deafness in 1 confirmed-deaf horse and 2 suspected-deaf horses that did not have the EDNBR gene mutation.

The higher prevalence of the EDNBR gene mutation among confirmed- and suspected-deaf horses, compared with the prevalence in the APHA population as a whole, could have been attributable to the fact that only 29% of APHA-registered horses have an overo or tovero pattern (patterns most strongly associated with OLWFS), whereas all horses in the present study had one of those patterns.[11,12,16] One study[15] revealed that only 10% of tobiano horses were heterozygous for the

EDNBR gene mutation, whereas 71% of all overo, tovero, white, and overo-blend horses combined were heterozygous for the mutation. In that study, when frame and frame-blend horses were considered separately, 96% were heterozygous, whereas 12% of splashed white overo horses were heterozygous. In the present study, the sole confirmed-deaf horse that did not have the EDNBR gene mutation was phenotypically a loud (> 50% white) splashed white overo stallion that was possibly homozygous for this overo pattern because 33 of its 36 offspring had a minimally expressed splashed white overo phenotype and 2 foals had a tovero phenotype. Additionally, all types of overo horses include those that are heterozygous for the EDNBR gene mutation, including 95% to 100% of frame overo horses, 12% of splashed white overo horses, 20% of sabino overo horses, and 58% of tovero horses.[13,15] Therefore, the presence of the mutation alone does not necessarily result in deafness. Because 3 of the nondeaf horses were also heterozygous for the mutation, the relationship between the mutation and deafness could represent 2 independent genes that are prevalent in certain overo horses. Additional investigation of the genetics of deafness in horses is warranted.

Deafness has implications for the management and training of affected horses. In our experience, mistreatment of horses can occur when handlers or trainers do not recognize the deafness and become frustrated from a lack of response to verbal cues. With recognition of deafness, owners can successfully train horses with visual and tactile cues. All confirmed-deaf horses of riding age (n = 5), with the exception of the ataxic horse, were successful riding, show, or performance horses. In addition, most suspected-deaf horses were being used as intended, which suggested that deafness does not necessarily result in poor performance in affected horses.

Veterinarians should be aware that sensorineural deafness may exist in some but not all splashed white overo, frame overo, tovero, and overo-blend Paint horses, particularly those with large amounts of white on the face and limbs and those with partially heterochromic or completely blue eyes. This awareness is particularly relevant when performing prepurchase examinations and counseling owners about management and handling of deaf horses as well as the potential heritability of the trait.

a. Nicolet Viking IV, Nicolet Biomedical Inc, Madison, Wis.
b. Nihon Kohden Neuropack 4, Nihon Kohden America Inc, Foothill Ranch, Calif.

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Appendix appears on the next page.

Appendix

Definitions of phenotypic classes used to describe coat color patterns in American Paint Horses.

Pattern	Nature of white spotting	Nature of white edges	Presence of white crossing dorsal midline	Presence of white on limbs	Head markings	Tail color	Eye color	Other feature
Tobiano	Distinct as oval or round pattern; flank and chest often dark color	Crisp	Often	Usually on all 4 limbs	Similar to solid-color horses (blaze, strip, star, or none)	Often 2-colored (base color and white)	Variable	NA
Frame overo	White spots arranged horizontally on neck and trunk and surrounded by base color	Jagged	Never	Usually similar to solid-color horses, with dark limbs or small amount of white (eg, "socks") and white not usually extending above the carpus or tarsus	Often a large amount (blaze, bald, apron, or bonnet)	One color (dark)	Commonly blue	White body markings do not usually connect with limb markings
Splashed white overo	White on ventrum, giving appearance of having been dipped in white	Crisp	Only in horses with large amount of white on trunk	3 or more; usually all 4	Large amount (apron or bonnet)	Often small area of white hair; otherwise dark	Commonly blue	White extends above limbs, often connecting to spots on trunk or abdomen
Sabino overo	White extends from coronet up to the ventral abdomen, often as belly spots with roan hair	Flecked or roan edges	Only in homozygous sabino horses, which are completely or nearly completely white	2 or more; often all 4	Large amount, but less than splashed overo (blaze to bald most common)	Dark	Variable	Completely or nearly completely white phenotype in homozygous horses
Tovero	Cross between tobiano and one of the overo patterns	Depends on underlying overo pattern	Usually	Usually all 4	Usually large amount (bald, apron, bonnet, or medicine hat)	Often 2-colored	Variable	Usually mostly white, often with only small amounts of dark on the head and in tail

NA = Not applicable.