Multiple ocular developmental defects in four closely related alpacas

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Abstract

Objective To describe the clinical, gross pathologic, and histopathologic findings for a visually impaired 5.8-year-old female alpaca with multiple ocular abnormalities, as well as the clinical findings for three closely related alpacas.

Animals studied Four alpacas.

 Procedures Ophthalmic examination was performed on a 16-month-old female alpaca following observation of visual impairment while hospitalized for an unrelated illness. Following acute systemic decline and death 4.5 years later, the alpaca’s brain, optic nerves, and eyes were examined grossly and histologically. Ophthalmic examination of three closely related alpacas was subsequently performed.

 Results The 16-month-old female alpaca (Alpaca 1) had ophthalmoscopic findings suggestive of a coloboma or hypoplasia of the retinal pigment epithelium (RPE) and choroid, and suspected optic nerve hypoplasia OU. Histopathology performed 4.5 years later revealed moderate to severe choroidal, RPE, and retinal hypoplasia with multifocal retinal detachments OU. However, the optic nerves were normal in size and histologic appearance when compared to an age-matched control. Clinical evaluation of the 2-year-old son of Alpaca 1 revealed iris colobomata OU and choroidal dysplasia/hypoplasia OD in addition to nonpathologic variations in melanin density including heterochromia iridis and a subalbinotic fundus OU. Clinical evaluation of the 13-year-old mother of Alpaca 1 revealed heterochromia iridis, cataracts, and a subalbinotic fundus OU. A 2-year-old half-brother of Alpaca 1 had an RPE and choroidal coloboma OS.

Conclusion The developmental ocular abnormalities diagnosed in these closely related alpacas are likely hereditary.

Key Words: camelid, coloboma, developmental, embryology, hereditary, ocular dysgenesis

INTRODUCTION

Few congenital uveal and posterior segment defects have been described in living camelids, though histopathologic reports are more numerous.1–4 While thoroughly described in other species including cattle and dogs, colobomata as heritable defects have yet to be demonstrated in camelids.5–7 Therefore, the purpose of this study was to describe the initial clinical ophthalmic examination findings and subsequent gross pathologic and histopathologic findings of an alpaca with multiple ocular abnormalities and signs of visual impairment. Also described are the pedigree analysis and clinical ophthalmic examination findings of three alpacas closely related to the initial patient evaluated.

CASE PRESENTATION

A 16-month-old female alpaca of medium brown coloration (Alpaca 1) was referred to the University of California Veterinary Medical Teaching Hospital (UCD
VMTH) for assessment of a suspected bone sequestrum in the left femur. General physical examination revealed purulent discharge from numerous tracts in the skin over a soft tissue swelling on the medial aspect of her left thigh. The cria was noted to walk into fixed objects. Past pertinent history included a mid-diaphyseal fracture of the left femur which had been diagnosed at the UCD VMTH when the animal was 6 weeks old. Behavioral observations regarding apparent vision and results of an ophthalmic examination were not reported at that original visit. The fracture had been treated with strict confinement for 60 days and intramuscular administration of 0.5 mg/kg ketoprofen every 12 h for 10 days following which the cria apparently regained adequate limb function and remained apparently healthy until the draining tracts were noted. Radiographs at the follow-up visit confirmed that the fracture had healed but that there were two cortical bone sequestra adjacent to the fracture site. However, there was behavioral evidence of visual impairment, therefore ophthalmic consultation was sought.

Upon questioning, the owners did not report having noticed any evidence of visual deficits but acknowledged that the alpaca had always been kept in surroundings with which she was familiar. In photopic conditions, the alpaca was noted to bump into several fixed objects. Although behavioral assessment of vision can be difficult in any young alpaca and, in this case, evaluation was further complicated by orthopedic dysfunction, the examining clinicians interpreted the behavior as resulting from visual impairment. Visual behavior was not assessed under scotopic conditions. On ophthalmic examination, both eyes were open and appeared to be comfortable. There was no ocular discharge or facial asymmetry. No anisocoria was noted; however, both pupils were considered abnormally dilated for the ambient light conditions. Direct and consensual pupillary light reflexes (PLRs) were present but slow and incomplete OU. In both eyes, the menace response was absent and dazzle reflex was inconsistent, but the palpebral reflex was complete. No abnormalities in globe position or movements were noted OU.

Ophthalmic examination included slit lamp biomicroscopy before and after pupil dilation, and binocular indirect ophthalmoscopy following pupil dilation achieved with topical application of tropicamide. The eyelids, third eyelid, conjunctiva, cornea, anterior chamber, iris, lens, and vitreous were within normal limits OU. Both optic nerve heads were considered symmetrically smaller than normal but they were not depressed. In each eye, there was a geographic region of the fundus dorsal to the optic nerve head where reduced density of retinal pigment epithelium (RPE) and choroidal melanin permitted visualization of choroidal vessels. The area of choroidal vessel exposure was larger in the left eye than the right. The rest of the fundus examination findings were considered to be within normal limits OU. Aqueous tear production, intraocular pressure, and corneal fluorescein retention were not assessed. The clinical diagnoses were optic nerve hypoplasia OU, and RPE and choroidal colobomata OU. Optic nerve hypoplasia was the suspected cause of visual impairment while the colobomata were considered incidental findings. Diagnostic testing to better anatomically localize the visual deficit including electroretinogram, ocular ultrasound, optical coherence tomography, computed tomography, or magnetic resonance imaging was not pursued. Following successful removal of the bony sequestra under general anesthesia, the alpaca was discharged to the care of the owners who were advised, because of the ocular defects, not to use her for breeding.

The owners reported that Alpaca 1 experienced no apparent health concerns following recovery from sequestra removal until approximately 4.5 years later when they found her acutely ill. She was weak, unable to rise, and held her neck and legs extended. Her body temperature was 34.4 °C (reference range: 37.5 °C–38.9 °C). She died without treatment and a postmortem examination was performed by the local veterinarian approximately 4 h later. Necropsy showed several gross changes suggestive of sepsis, though a definitive cause of death was not identified. At the owners’ request, the veterinarian removed the alpaca’s head during the postmortem examination and it was stored in a freezer at approximately −20 °C for 4 days before being submitted on ice to the UCD VMTH Pathology Service for histopathologic examination. Prior to gross dissection, the head was allowed to thaw at room temperature. The entire brain, along with the optic nerves, and both eyes were removed en bloc and were considered grossly normal. They were fixed whole in 10% neutral buffered formalin for approximately 2 weeks prior to sectioning for histopathologic evaluation. Following fixation, the horizontal diameter of each optic nerve was measured at two points: immediately as it exited the sclera and 8.0 mm caudal to the sclera. Horizontal optic nerve diameters immediately behind the right and left eyes were 5.0 and 4.5 mm, respectively. Eight mm caudal to the posterior pole they were 5.0 mm in diameter OU. A globe from an approximately age-matched (5-year-old) male alpaca with no history of ocular disease was fixed and measured in the same manner. The horizontal diameter of the optic nerve was larger than that of the Alpaca 1 at the level of the globe (6.0 mm OU), but was only 4.0 mm diameter when measured at the point 8 mm caudal to the sclera.

Both optic nerves from Alpaca 1 and one optic nerve from the normal, age-matched alpaca were cross-sectioned about 8 mm caudal to the globe for histopathologic examination. Additionally, an approximately 10-mm longitudinal section of each nerve was taken just caudal to the region examined in cross section. These tissues were processed routinely, embedded in paraffin, sectioned into 4-μM slices, mounted, and stained with hematoxylin and cosin (H&E), Masson’s trichrome (to semiquantify connective tissue septa relative to intervening parenchyma),
and Bodian’s stain (to selectively stain nerve axons). The optic nerves from Alpaca 1 were histologically similar to those from the control alpaca. Examination of Masson’s trichrome-stained sections of both optic nerves from Alpaca 1 and one nerve from the control alpaca confirmed that all three had similar amounts of pial collagen. Staining intensity with Bodian’s stain was similar for both alpacas. Hematoxylin and eosin-stained sections of the optic tract, optic chiasm, and occipital cortex from Alpaca 1 were slightly distorted by autolysis and mild freezing artifact, but otherwise were histologically unremarkable.

Both globes of Alpaca 1 were then examined grossly and histologically. To facilitate this, they were sectioned along two parallel vertical meridians so as to create an approximately 1.5 cm central section of each globe which included the optic nerve. Gross examination revealed no observable differences in optic disk sizes between the right and left eyes of Alpaca 1. On cut section of the right eye, a 9-mm × 3-mm region of reduced pigment density was present OD, approximately 4 mm dorsal to the optic nerve head. The left eye had a similar but larger triangular wedge of reduced pigment density, comprising about 30% of the central dorsal fundus (Fig. 1). Globe sections were routinely processed, embedded in paraffin, sectioned into 4-μM slices, and stained with H&E. The eye from the age-matched normal alpaca was fixed and sectioned in the same manner and confirmed to be histologically normal.

Histologic examination of multiple sections from several regions of both globes from Alpaca 1 revealed diffuse but focally severe choroidal thinning with multifocal, regionally extensive hypopigmentation (Fig. 2). Multifocal areas of reduced numbers of RPE cells were seen OU, and in some areas RPE cells were absent. The overlying retina was segmentally atrophic and disorganized with thinned nerve fiber and ganglion cell layers, fusion of the inner and outer nuclear layers, and nearly complete absence of photoreceptors OU. The right globe had a perineural region of thinned retina having a markedly thin nerve fiber layer, fused and disorganized nuclear layers, rare ganglion cells, and few photoreceptor segments (Fig. 2). Multifocal regions of chorioretinal scarring with intervening retinal separation and associated RPE hypertrophy, as well as areas devoid of RPE pigment, were seen OD (Fig. 3). Otherwise, both globes were histologically normal except for small foci of cortical lens fiber degeneration OD and mild, focal limbal scleritis OU, the latter of which was likely explained by the animal’s systemic illness. The histologic diagnoses were moderate to severe choroidal, RPE, and retinal hypoplasia with multifocal retinal detachments OU.

To better determine a possible cause for the abnormalities noted in Alpaca 1, three closely related alpacas were examined clinically. These included an approximately 2-year-old, dark silver-gray male (Alpaca 2) identified as the son of Alpaca 1, an approximately 13-year-old, medium silver-gray female (Alpaca 3) identified as the mother of Alpaca 1 (and of Alpaca 4), and an approximately 2-year-old, medium brown male (Alpaca 4) identified as a half-brother of Alpaca 1. More specific pedigree information was provided by the owners (Fig. 4). The owners believed that Alpacas 3 and 4 had normal vision but suspected that Alpaca 2 (son) had visual deficits but was not completely blind. They based this upon the observation that he approached unknown objects very closely and then appeared to be suddenly startled by them and backed away. They also reported that when he found himself separated from the herd, he tended to run back to where he had last been with them rather than visually seeking them before or while moving.

Each of these three alpacas underwent ophthalmic examination as described for Alpaca 1. In all three animals, both eyes were open and appeared comfortable, there was no ocular discharge or facial asymmetry, and the menace response and dazzle and palpebral reflexes were present and complete OU. Their globe positions and movements were normal OU, and all alpacas behaved as if sighted in photopic conditions. Anisocoria was not noted in any alpaca. Direct and consensual PLRs were brisk and complete OU in Alpacas 2 (son) and 3 (mother); however, they were slower and less complete OU in Alpaca 4 (half-brother). This was attributed to increased sympathetic tone as this alpaca appeared fearful throughout the examination. Intraocular pressure, as estimated by applanation tonometry, was within normal limits OU for all three alpacas.8

Figure 1. Gross images of central regions of the formalin-fixed right (a) and left (b) globes of a 5.8-year-old female alpaca (Alpaca 1). Note the 9-mm × 3-mm region of reduced fundic melanin density dorsal to the optic disk OD (a; arrow) and the large region of reduced fundic melanin density comprising ~30% of the central dorsal fundus OS (b; arrow).
Slit lamp biomicroscopy before and after pupil dilation, and binocular indirect ophthalmoscopy following pupil dilation with topical application of tropicamide revealed no ocular lesions except for suspected developmental abnormalities of the irides and choroid OU of Alpaca 2 (son), both lenses of Alpaca 3 (mother), and the choroid and RPE OS of Alpaca 4 (half-brother). Specifically, Alpaca 2 (son) had generalized heterochromia iridis with focal regions of thinned or absent iridal stroma at the ‘6 o’clock’ position ventral to the pupillary margin OU. These comprised a single region approximately 4- to 5-mm-diameter OS and two conjoined regions each approximately 5- to 6-mm-diameter OD; posterior iris epithelium protruded through all three regions (Fig. 5). These were all thought to be iris colobomata. Both fundi of Alpaca 2 were subalbinotic. The fundus OS was considered to be within normal limits, however, the fundus OD had generalized, mildly dysplastic choroidal vasculature in the superior central and mid-peripheral fundus, and a well-demarcated, horizontally oval, flat region of hypoplastic choroid approximately three optic disk diameters (ODDs) superior to the optic nerve head (Fig. 6). This region was estimated as 1 ODD × 2 ODDs in size. The clinical diagnoses for Alpaca 2 were typical iris coloboma OU and choroidal dysplasia and hypoplasia OD. This animal did not show evidence of visual deficits in either eye and the clinical diagnoses were not interpreted as affecting vision, suggesting that the abnormal tendencies appreciated by the owner may have been behavioral. Alpaca 3 (mother) had generalized heterochromia iridis and the lenses displayed focal, prominent anterior suture lines and posterior nuclear fibrillar cataracts, interpreted as age-related (Fig. 7). A subalbinotic fundus was also present OU. Alpaca 4 (half-brother) had heavily melanotic irides. Choroidal and RPE melanin prevented examination of choroidal vessels OU. However, in the left fundus, there was a well-demarcated, flat, oval, poorly melanotic region (approximately ¼ ODD in diameter) in the far peripheral temporal fundus (Fig. 8). This was diagnosed as an RPE and choroidal coloboma OS.

**DISCUSSION**

In this case series, we document the clinical and pathologic findings for a visually impaired alpaca with presumed developmental ocular defects, as well as clinical findings for three closely related alpacas. Although developmental ocular defects have been documented in camels,1–3,9,10 reports are infrequent, and to the authors’ knowledge, the present series is the first to report such findings in related camels. To date, reports of developmental defects in camelid eyes and adnexa have described largely abnormalities of the nasolacrimal system or anterior uvea, however, camelids with presumed developmental defects of the posterior segment have also been reported.2–4,10,11 These include case reports of a llama with optic nerve hypoplasia, an unspecified coloboma, and retinal dysplasia;2 a unilateral large coloboma near the optic disk of an adult llama;3 an optic nerve coloboma in a South American alpaca;4 peripapillary colobomata, retinal dysplasia, and
microphthalmia in various combinations in a number of alpaca crias less than 8 weeks of age\textsuperscript{10}; and retinal separation, vitreous fibrosis and ossification, optic nerve hypoplasia, tunica vasculosa lentis, and retinal aplasia in a camelid cria.\textsuperscript{10}

The small number of animals examined in the present study did not permit the identification of a specific cause of the ocular defects. However, the fact that the 4 camels in this report were closely related suggests a possible heritable cause for the ocular defects. The uveal defects were multigenerational, consistent with familial inheritance. However, the ocular phenotypes of the remaining alpacas presented in the pedigree (Fig. 4) are unknown, and many more animals would need to be examined to establish a more specific inheritance pattern. Due to concerns regarding a potential genetic cause for the ocular defects seen in Alpaca 1, the owners were advised against breeding this individual or repeating the breeding that produced Alpaca 1. Certainly, posterior segment colobomata, such as in three of the four alpacas of the current report are believed to be inherited in some breeds of cattle\textsuperscript{6} and dogs.\textsuperscript{12,13} A 1972 report of colobomata in Charolais cattle described bilateral typical colobomata with respect to their position on the optic disk; their sizes ranged widely, and occasionally they affected the entire optic disk.\textsuperscript{6} Genetic studies suggested a dominant mode of inheritance with variable expressivity.\textsuperscript{7} In dogs, ocular posterior segment colobomata can be seen alone or as a component of Collie eye anomaly (CEA).\textsuperscript{14} An intronic deletion of a 7.8-kb area of the \textit{NHEJ1} gene located on canine chromosome number 37 has been identified as a marker for this disease.\textsuperscript{12,13,15} There are multiple phenotypic manifestations of CEA. Some affected dogs are blind due to secondary retinal separation or nonattachment, while some have posterior segment abnormalities that are visible ophthalmoscopically but the dogs have apparently normal vision.\textsuperscript{13} The characteristic histologic lesion is choroidal hypoplasia in which the choroid is thin and has little or no melanin.\textsuperscript{14,15} In conjunction with this defect, the RPE is poorly pigmented and there may be

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure4.png}
\caption{Pedigree showing the relationships among the 4 alpacas examined. Circles represent females; squares represent males. The individual’s coat color is described inside each shape and the examined alpacas are assigned a number (1–4).}
\end{figure}

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure5.png}
\caption{External photographs of the right (a) and left (b) eyes of an approximately 2-year-old, dark silver-gray male (Alpaca 2) identified as the son of Alpaca 1. Note the generalized heterochromia iridis and regional iridal stromal absence OU interpreted as two conjoined iridal colobomata each ~5- to 6-mm-diameter OD (a) and a 4- to 5-mm-diameter iridal coloboma OS (b). The white approximately parallel lines apparently within the coloboma OD are corneal reflections of the periorcular hair/cilia.}
\end{figure}
colobomata of variable size. If the coloboma is large, retinal separation may be present as a secondary lesion. The similarity of the histologic lesions seen in CEA to those that we report here in Alpaca 1 may suggest a similar embryologic defect may have occurred.

Three of the four alpacas of this report (Alpacas 2, 3, and 4) were of a silver-gray color. Although the genetics determining coat color in alpacas remains largely unknown, it is possible that (as in other species) a dilution gene is responsible for colors such as silver and gray. Given the confirmed association of ocular developmental defects in color-dilute phenotypes in other species, it is possible that the amount of eumelanin or pheomelanin present, or a mutation linked to a gene responsible for coat color may be involved in the pathogenesis of the lesions in these alpacas. Color variation in the iris and fundus of alpacas is common and appears to be related to coat color. Lightly pigmented alpacas tend to have irides composed of some combination of gray, blue, brown, and white, and have reduced fundic melanin compared to alpacas with dark coats. Three variations of fundic pigmentation have been described and include complete pigmentation (in black, red/brown, and brown alpacas), light pigmentation (in white and lightly pigmented alpacas), and often areas that lack pigment in the dorsal fundus (in light tan or gray with white leg markings).

![Figure 6](image6.png)

**Figure 6.** Funduscopic image of the superior region of the right eye of the approximately 2-year-old, dark silver-gray male (Alpaca 2; son of Alpaca 1) shown in Figure 5a. Note the well-demarcated, horizontally ovoid, nonraised region of hypoplastic choroid (arrows) superior to the optic nerve head.

![Figure 7](image7.png)

**Figure 7.** External photographs of the right (a) and left (b) eyes of an approximately 13-year-old, medium silver-gray female (Alpaca 3) identified as the mother of Alpaca 1 and Alpaca 4. Note the generalized heterochromia iridis and the focal anterior suture line cataracts. Posterior nuclear fibrillar cataracts were also evident on slit lamp biomicroscopy.

![Figure 8](image8.png)

**Figure 8.** Funduscopic image of the right eye of an approximately 2-year-old, medium brown male (Alpaca 4) identified as a half-brother of Alpaca 1. Note that dense choroidal and retinal pigment epithelium (RPE) melanin precludes examination of the choroidal vessels; however, a well-demarcated, nonraised region of relative melanin absence interpreted as an RPE coloboma about ¼ optic disk diameter in size was present in the peripheral temporal fundus.
Likewise, merle ocular dysgenesis (MOD) represents an ocular disease linked to coat color. This is an inherited disease of Australian Shepherd and other dogs that are homozygous dominant for the merle gene. Affected animals have multiple ocular defects including colobomata of the uvea, retina, and sclera; microphthalmia; cataracts; and retinal dysplasia, separation, or both; they may also be deaf. Embryologic studies have shown that the atypical colobomata seen with MOD are a result of dysplasia of the outer layer of the optic cup, which in normal development goes on to become the RPE. Proper development of the optic cup induces normal differentiation of the choroid and sclera as well as the intrinsic pupillary muscles, explaining the location and extent of these atypical colobomata. While the genetic basis for MOD is associated with the merle gene, amelanosis does not appear to be a direct cause of the ocular abnormalities. The lesions seen in MOD cause varying degrees of visual impairment. The clinical significance of the colobomata in Alpacas 1, 2, and 4 remains unclear, especially as diffuse ocular disease was evident histopathologically in Alpaca 1.

Alpaca 1 had overt behavioral evidence of visual impairment when hospitalized at 16 months of age. In addition, mydriasis in ambient light, slow and incomplete PLRs, absent menace responses, and inconsistent dazzle reflexes OU suggested that she had markedly reduced light perception but not complete blindness. Based upon clinical findings at this visit, optic nerve hypoplasia was believed to be the cause of the visual impairment. However, histopathologic assessment 4.5 years later failed to confirm the presence of optic nerve hypoplasia and instead revealed chronic retinal degeneration. It is possible that retinal degeneration was present (although possibly not as advanced) at the time of initial examination. If so, it is possible that a number of features of the camelid fundus in general, as well as those of this particular individual rendered observation of the retinal degeneration more challenging. These include the absence of a tapetum, presence of very prominent retinal blood vessels, and (in Alpaca 1) regional hypomelanosis of the fundus. Unfortunately, diagnostic tests such as electroretinography, visual evoked potentials, and retinal optical coherence tomography were not performed at the first visit, and repeat ophthalmic examinations were not sought by the owners during the 4.5 years between this examination and the alpaca’s death. However, given the subjectivity of vision assessment in this species, especially by owners, and the need to anatomically localize the lesion within the visual system of Alpaca 1, electroretinography may have been particularly useful. Dogs confirmed to have optic nerve hypoplasia were shown in one study to have an absent a-wave, reduced b-wave, and low flicker-fusion frequency. However, this abnormal waveform may have been confounded by any retinal degeneration present in Alpaca 1 at the time of testing. It is also interesting to consider the role of the RPE and choroidal colobomata in Alpaca 1’s visual deficit.

Evaluation of horses with similar colobomata as a sole finding did not reveal significant visual field deficits. However, in Alpaca 1 the colobomata were located just dorsal to the optic nerve head, likely falling within the visual streak and potentially increasing the likelihood of a partial visual deficit. It is likely that the owners had not noted any signs of visual impairment in the first 16 months of Alpaca 1’s life because alpacas, as herd animals, rely heavily on other members of the herd and on familiarity with their surroundings. Thus, blind animals may develop otherwise normally and not show signs of visual impairment until they are placed in a novel environment. Because ocular defects suspected to be hereditary occur in this species, and given the potential association between anterior segment abnormalities and vision-threatening posterior segment abnormalities, prebreeding ophthalmic examinations are recommended until the genetics of these lesions are more fully elucidated.

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