Ophthalmologic and oculopathologic findings in red-tailed hawks and Cooper's hawks with naturally acquired West Nile virus infection

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Objective—To assess ophthalmologic features and ocular lesions in red-tailed hawks and Cooper's hawks naturally infected with West Nile virus (WNV).

Design—Original study.

Animals—13 hawks.

Procedures—All hawks underwent complete ophthalmic examinations including slit lamp biomicroscopy and binocular indirect ophthalmoscopy. Eleven hawks were euthanized because of a grave prognosis; complete necropsies were performed. Eyes, brain, heart, and kidneys were processed for histologic and immunohistochemical examinations. Pooled tissue homogenates and aqueous humor samples were assessed for WNV nucleic acid via PCR assay, and anti-WNV antibody titers in aqueous humor and plasma were determined.

Results—All birds had similar funduscopic abnormalities including exudative chorioretinal lesions and chorioretinal scarring in a geographic or linear pattern. Eleven birds were euthanized, and 2 birds were released. Plasma from both released hawks and plasma and aqueous humor of all euthanized hawks that were evaluated contained anti-WNV antibodies. Except for 1 hawk, all euthanized hawks had WNV-associated disease (determined via detection of WNV antigen or nucleic acid in at least 1 organ). Histopathologic ocular abnormalities, most commonly pectenitis, were detected in all euthanized birds; several birds had segmental choroiditis, often with corresponding segmental retinal atrophy. West Nile virus antigen was detected in the retinas of 9 of the euthanized birds. In 2 hawks, WNV antigen was detected in the retina only.

Conclusions and Clinical Relevance—Results indicated that funduscopically detectable chorioretinal lesions appear to be associated with WNV disease in hawks. Detection of ocular lesions may aid in antemortem or postmortem diagnosis of this condition. (*J Am Vet Med Assoc* 2007;231:1240–1248)

West Nile virus is a flavivirus of the Japanese encephalitis antigen complex that has been associated with considerable death rates among various species of birds of prey since its introduction to North America in 1999.[1–7] Birds are the natural host for the virus, and mosquitoes are the primary vectors responsible for spreading infection to humans and other animals. Visual impairment and inflammation of the pecten and choroid as a result of WNV infection have been detected in various hawk species such as red-tailed hawks (*Buteo jamaicensis*), Cooper's hawks (*Accipiter cooperi*), and goshawks (*Accipiter gentilis*).[4,6] In 1 study,[4] histologic ocular lesions including pectenitis, multifocal lymphoplasmacytic choroiditis, and iridocyclitis were evident in as many as 7 of 11 WNV-infected hawks. Within the eyes of those birds, WNV antigen was limited to the retina. Ocular lesions including optic neuritis, anterior uveitis, vitritis, and chorioretinitis have also been identified in humans infected with WNV.[8–12] To our knowledge, no study to date has described fundic lesions associated with WNV infection in birds. The purpose of the study reported here was to assess ophthalmologic features and ocular lesions and evaluate their possible associations with serologic and virologic findings in red-tailed hawks and Cooper's hawks that were naturally infected with WNV.

Abbreviations

WNV	West Nile virus
SLE	Saint Louis encephalitis
PRNT	Plaque reduction neutralization test
CCM	Cell culture medium
RT	Reverse transcription
RPE	Retinal pigmented epithelium

Materials and Methods

Selection criteria—From May through November 2005, 10 red-tailed hawks and 3 Cooper's hawks that were

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admitted to The Raptor Center of the University of Minnesota were suspected of having WNV disease. During ophthalmologic examination, funduscopic lesions were detected, and these birds were included in the study (designated as birds 1 though 13). Fourteen other red-tailed hawks were submitted to The Raptor Center during the same time period and underwent ophthalmic examinations. However, those hawks were not suspected to be infected with WNV on the basis of adequate nutritional state and lack of neurologic signs. Some data collected from these birds were used, but the birds were not the focus of the study.

Clinical assessment—Birds underwent a complete physical examination, CBC, and full-body radiography. The approximate age of the birds was determined by the clinicians who evaluated the plumage on the basis of published criteria.[13,14] Slit lamp biomicroscopy[a] and binocular indirect ophthalmoscopy[b] were performed; fundic photographs[c] were obtained to record abnormalities. Fundic lesions were subjectively graded as mild (≤ 25% of the fundus affected), moderate (26% to 50% of the fundus affected), or severe (> 50% of the fundus affected). Approximately 1 mL of blood was collected from the ulnar vein into syringes containing heparin for serologic testing for anti-WNV antibodies. Blood samples were centrifuged, and plasma was separated and stored at –20°C until used for serologic testing. When the prognosis was grave, birds were euthanized by use of an IV injection of euthanasia solution at the discretion of the clinician. The carcasses were submitted to the Veterinary Diagnostic Laboratory of the University of Minnesota for necropsy.

Collection of tissues—Necropsies were performed within 1 to 2 hours after euthanasia. The left and right eye and samples of brain (cerebrum and cerebellum), heart, kidneys, and spleen were fixed in neutral-buffered 10% formalin for histologic and immunohistochemical examinations. Aqueous humor was collected immediately after death from 1 eye of each of the red-tailed hawks and from both eyes of the Cooper's hawks, which are considerably smaller than red-tailed hawks, whenever possible. Samples of aqueous humor were submitted for WNV nucleic acid–specific PCR assay, assessment of anti-WNV and anti-SLE virus antibody titers via PRNTs, and virus isolation.

Histologic and immunohistochemical examinations—Formalin-fixed tissues were embedded in paraffin. Sections (4 µm thick) were cut and stained with H&E. The degree of inflammation in the eyes was subjectively graded as absent, mild, moderate, or marked on the basis of the estimated number of infiltrating lymphocytes, plasma cells, and macrophages. Two sections per eye were evaluated via light microscopy to characterize the degree of inflammation. Sections of several eyes were stained with von Kossa stain to determine whether retinal calcification was present. A peroxidase-based polymer system[d] was used for immunohistochemical detection of WNV antigen in the collected organ specimens.[4] The amount of antigen expression in the eyes was described as absent (negative), mild (+), moderate (++), or marked (+++) on the basis of the estimated number of WNV antigen–positive cells.[4] Two sections per eye were evaluated to determine the amount of antigen expression.

Serologic testing—Samples of aqueous humor from 9 of the birds and plasma from 8 of the birds were assessed for anti-WNV antibody via PRNTs.[e] Briefly, plasma or aqueous humor samples were serially diluted (2-fold dilutions) from 1:20 to 1:640 in a 0.1-mL volume of CCM (minimum essential medium with Earle's salts,[f] 10% fetal bovine serum, and ciprofloxacin hydrochloride[g] [10 µg/mL]). Approximately 200 plaque-forming units of WNV or SLE virus were added in a 0.1-mL volume of CCM containing 10% guinea pig complement[h] to each dilution and incubated for 1 hour at 37°C in a 5% carbon dioxide incubator. One hundred microliters of the virus-containing suspension was overlaid onto a confluent monolayer of Vero cells and incubated for an additional hour under the aforementioned conditions. Cell monolayers were overlaid with CCM containing 2% fetal bovine serum and 1% low–melting-point agarose.[i] Assays were incubated for 3 days (WNV) or 6 days (SLE virus) under the aforementioned conditions. Monolayers were stained overnight (15 to 18 hours) by adding 3 drops of CCM containing 3 mg of neutral red/mL.[j] Plaques were counted on day 4 (WNV) or day 7 (SLE virus). Wells were scored as positive for neutralization if the number of plaques was greater than or equal to the mean plaque count at a 1:10 dilution of input virus.

RT-PCR assay—The aqueous humor of 9 birds and pooled tissue samples including heart, kidneys, and brain (cerebrum and cerebellum) of 8 birds were examined for WNV via RT-PCR assay.[e] In addition, brain samples of 10 birds were examined for WNV via RT-PCR assay at the Minnesota Department of Health as part of a statewide WNV monitoring program. Nucleic acid extraction and amplification were performed by use of the probes and protocol previously described by Lanciotti et al.[15] Reactions were performed with 2 µL of RNA in a 25-µL reaction. Amplification was done in a 2-stage reaction with RT at 42°C for 900 seconds and denaturation at 95°C for 600 seconds, followed by 39 cycles of 95°C for 15 seconds, 50°C for 10 seconds, and 60°C for 100 seconds.

Virus isolation—Samples of aqueous humor from 8 birds and pooled tissue samples including heart, kidneys, and brain (cerebrum and cerebellum) from 8 birds were examined for WNV via virus isolation.[e] Tissue homogenates (0.5 mL) and aqueous humor samples (0.1 mL) were inoculated onto subconfluent monolayers of Vero cells and incubated for 1 hour at 37°C in 5% carbon dioxide. Monolayers were rinsed with PBS solution, overlaid with 5 mL of CCM, and maintained as described previously. Monolayers were checked daily for cytopathic effect and were split 1:6 at 5 to 7 and 12 to 14 days. Virus was identified in cultures with cytopathic effect via RT-PCR assay as described.

For 2 birds (birds 3 and 10), aqueous humor was not submitted in sufficient quantity to perform all 3 tests. Therefore, antibody titer analysis (bird 3) and virus isolation (bird 10) were omitted in those instances.

Results

From June through November 2005, 10 red-tailed hawks and 3 Cooper's hawks were admitted to The Raptor Center of the University of Minnesota, and complete ophthalmic examinations revealed funduscopic lesions in each bird. These birds included 4 hatch-year red-tailed hawks, 2 immature red-tailed hawks (1 to 3 years of age), 4 adult red-tailed hawks, and 3 hatch-year Cooper's hawks. Of these, 8 red-tailed hawks and all 3 Cooper's

Table 1—Results of ophthalmologic examinations; assessments of plasma anti-WNV antibody titers; PCR assays for WNV nucleic acid; WNV isolation; and immunohistochemical (IHC) evaluations for WNV antigen in plasma, aqueous humor (AH), brain tissue, eyes, tissue homogenates, cerebrum (CB), cerebellum (CBL), kidneys (K), and heart in 10 red-tailed hawks (R) and 3 Cooper's hawks (C) suspected of having WNV infection.

	Anti-WNV antibody titer		PCR assay			Virus isolation		IHC			Ophthalmic findings	
Bird	Plasma	AH	AH	Tissue	Brain	AH	Tissue	Right eye	Left eye	Other organs with positive IHC results	Right eye	Left eye
1 (R)	ND	1:20	Pos	ND	Pos	Pos	ND	Neg	++	CB, CBL, K	CR scarring with linear exudates	Mild AU; CR scarring with linear and geographic exudates
2 (R)	> 1:640	1:160	Pos	Pos	Pos	Neg	Neg	+++	Neg	CB	Moderate AU; small CR scars	CR scarring with geographic exudates
3 (R)	> 1:640	ND	Neg	Pos	Pos	Neg	Neg	+++	Neg	None	Moderate AU; widespread CR scarring with geographic exudates	No abnormalities
4 (R)	1:160	1:20	Pos	Pos	Pos	Neg	Neg	++	+	CB, CBL, K	Mild AU; CR scarring with linear and geographic exudates	Mild AU; CR scarring with linear exudates
5 (R)	ND	1:80	Neg	Pos	Pos	Neg	Neg	Neg	Neg	Heart	Mild AU; CR scarring	No abnormalities
6 (R)	1:320	1:40	Pos	Pos	Pos	Neg	Neg	+	Neg	None	Mild AU; CR scarring with linear and geographic exudates	Mild AU; mild CR scarring
7 (R)	ND	1:160	Neg	Neg	Pos	Neg	Neg	Neg	+	Spleen	Mild AU; CR scarring with geographic exudates	Mild AU; CR scarring with small areas of exudates
8 (C)	ND	ND	ND	ND	Pos	ND	ND	Neg	++	CB, CBL	Punctate area of CR exudates	Linear CR exudates
9 (C)	> 1:640	1:160	Pos	Pos	Pos	Pos	Neg	++	Neg	CB, CBL	Mild AU; CR scarring with geographic exudates	Mild AU; CR scarring with geographic exudates
10 (C)	1:20	1:20	Neg	Pos	Pos	ND	Neg	++	+	K	CR scarring with linear and geographic exudates	CR scarring with linear exudates
11 (R)	1:320	NA	NA	NA	NA	NA	NA	NA	NA	NA	Small CR scars	Small CR scars with exudates
12 (R)	> 1:640	NA	NA	NA	NA	NA	NA	NA	NA	NA	Moderate AU; small CR scar with geographic exudates	Small peripheral CR scars
13 (R)	> 1:640	1:80	ND	ND	Neg	ND	ND	Neg	Neg	Neg	Mild AU; CR scar with large region of geographic exudates	Moderate AU with hyphema; fundus not visible

ND = Not done. Pos = Positive. Neg = Negative. IHC results = Absent (negative), mild (+), moderate (++), or marked (+++).
CR = Chorioretinal. AU = Anterior uveitis. NA = Not applicable.

hawks were euthanized because of a grave prognosis for survival and rehabilitation, and specimens were submitted for WNV testing; 2 adult red-tailed hawks were rehabilitated and released. In 7 of the 8 euthanized red-tailed hawks and the 3 Cooper's hawks, positive results were obtained for WNV nucleic acid and WNV antigen by use of PCR assay and immunohistochemical examination, respectively (**Table 1**). The remaining red-tailed hawk that was euthanized (bird 13) was seropositive for WNV, but results of WNV nucleic acid and WNV antigen testing via PCR assay and immunohistochemical examination, respectively, were negative. The 2 red-tailed hawks that were eventually released (birds 11 and 12) were seropositive for WNV, but assessments for WNV nucleic acid or WNV antigen were not performed.

In addition to the 13 birds in the study, 14 additional red-tailed hawks were admitted to The Raptor Center during the same time period and underwent ophthalmic examinations. Those hawks were not considered to be infected with WNV by the attending clinicians because of their adequate nutritional state and lack of neurologic signs. Ophthalmic examination of those 14 hawks revealed moderate anterior uveitis (n = 1 bird), mild focal to multifocal chorioretinal scarring (9), and mild to moderate vitreal hemorrhage (5). Samples of serum from 4 of those hawks were available for assess-

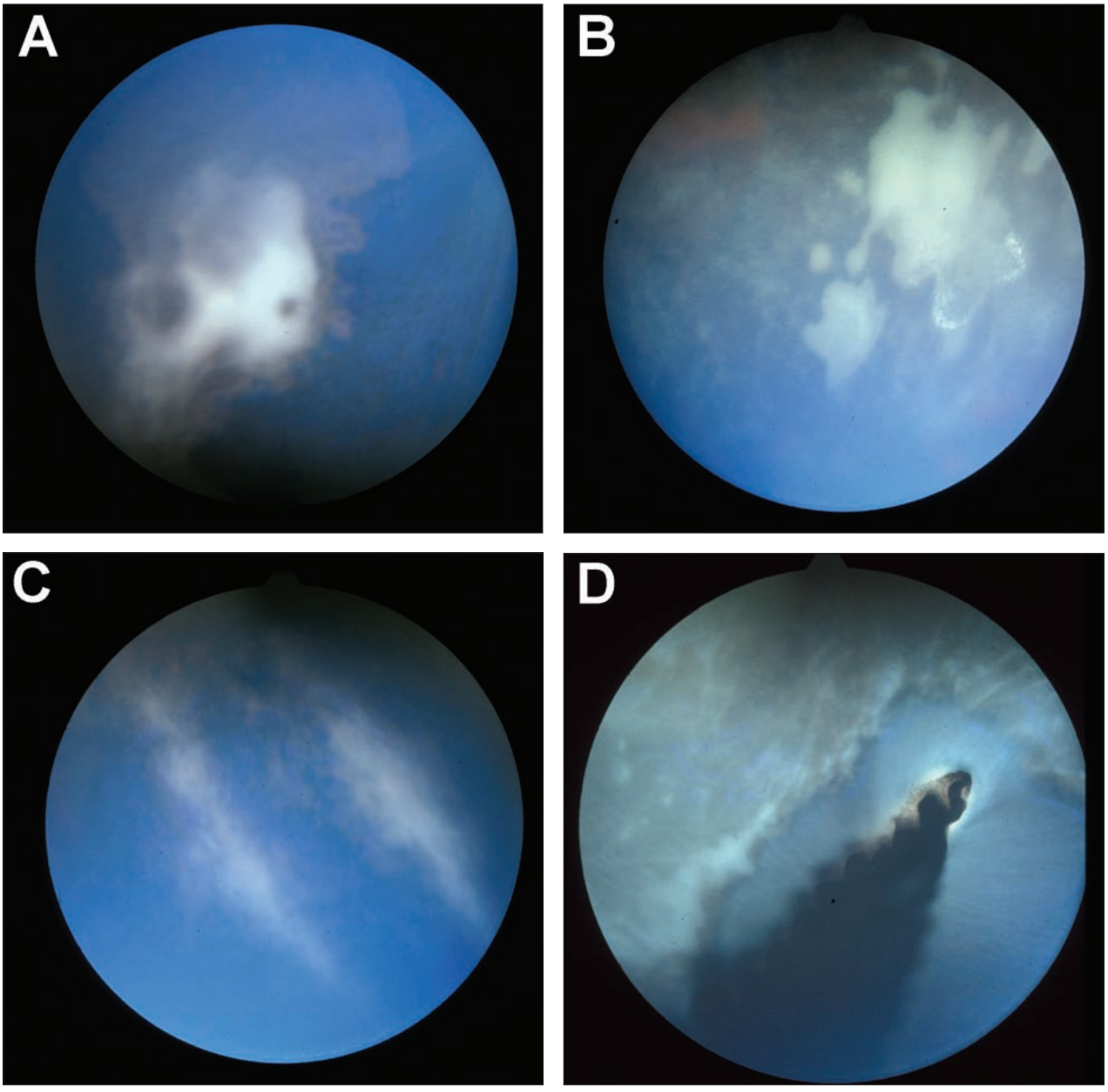

Figure 1—Photographs of a fundus of 3 red-tailed hawks and 1 Cooper's hawk that were infected with WNV. A—Fundus of a red-tailed hawk that has raised, geographic-shaped, chorioretinal exudates within a region of chorioretinal scarring. B—Fundus of a red-tailed hawk that has a geographic region of chorioretinal exudates with a rim of suspected mineralization. C—Fundus of a red-tailed hawk that has raised, linear, chorioretinal exudates. D—Fundus of a Cooper's hawk that has fine linear and punctate regions of chorioretinal exudates within a large region of chorioretinal scarring. The pecten is the heavily-pigmented structure; the retina immediately surrounding the pecten is of normal appearance for this species.

ment of anti-WNV antibody titer. All 4 hawks were seropositive, and antibody titers ranged from 1:20 to > 1:640. None of those hawks underwent postmortem examination. The fundic lesions in those hawks differed from the fundic lesions detected in the hawks of the study group.

Clinical and ophthalmic findings—Clinical signs at the initial examination included thin body condition (n = 13 birds), lethargy (5), neurologic abnormalities (including head tilt, tremors, ataxia, and disorientation; 4), vision loss (5), and a coracoid fracture (2). Clinicopathologic analyses were performed in 10 of the hawks. The results were within reference ranges in 2 birds and heterophilia (range, 19,795 to 43,687 heterophils/μL; reference range, 2,100 to 2,800 heterophils/μL[16]) was present in 8 birds. Radiographically, no abnormalities were detected in 7 birds; splenomegaly was evident in 4 birds, and a fractured coracoid was confirmed in 2 birds. Ophthalmic findings were similar in red-tailed hawks and Cooper's hawks. Ophthalmic examination revealed decreased vision in 5 of the 13 birds (determined from evidence of reduced tracking behavior or abnormal response when being caught from the cage). Three birds (4 eyes) had mydriatic pupils with absent pupillary light reflexes. There were corneal lesions consisting of keratic precipitates or corneal scarring in 4 birds (7 eyes). Mild to moderate anterior uveitis was detected in 10 birds (15 eyes). Fundic lesions were present in all 13 birds (23 eyes) and included a combination of active and inactive lesions. The fundus of the right eye of 1 red-tailed hawk was not visible because of hyphema (bird 13). By use of the grading system for fundic lesions, 10 eyes were considered mildly affected, 8 eyes were considered moderately affected, and 5 eyes were considered severely affected. Pectenitis, characterized by fibrinous material completely or partially coating the pecten, was detected in 6 eyes (5 birds). Twenty-one eyes (12 birds) had chorioretinal scarring that was characterized by flat, well-demarcated areas of retinal thinning, chorioretinal pallor, or pigment clumping (**Figure 1**). Fifteen eyes (11 birds) had active chorioretinitis, characterized by variably sized, raised, white lesions with indistinct margins. Thirteen eyes (8 birds) had chorioretinal lesions that were flat, well demarcated, bright white, and refractile and that were suspected to contain mineral. These potentially mineralized regions were present within an active-appearing lesion or within an area of chorioretinal scarring. The chorioretinal lesions were either geographic or linear in shape and often formed parallel lines or crisscrossing patterns.

Serologic findings—Samples of plasma obtained from 9 red-tailed hawks and 2 Cooper's hawks were assessed for anti-WNV antibodies (Table 1). Anti-WNV antibodies were detected in all birds; titers ranged from 1:20 to > 1:640. Anti-WNV antibody titers were also assessed in samples of aqueous humor that were collected after euthanasia from 7 red-tailed hawks and 2 Cooper's hawks; titers ranged from 1:20 to 1:160. The hawks were seronegative for anti-SLE virus antibodies, except for 1 bird (bird 13) that had a low anti-SLE virus antibody titer (1:20) and a high anti-WNV antibody titer (> 1:640).

PCR assay findings—A PCR assay was performed on samples of brain tissue from all euthanized hawks. West Nile virus nucleic acid was detected in brain tissue from 7 red-tailed hawks and 3 Cooper's hawks; 1 bird yielded negative results via PCR assay (Table 1). West Nile virus nucleic acid was detected in aqueous humor samples from 4 red-tailed hawks and 1 Cooper's hawk. In addition, tissue homogenates from 5 red-tailed hawks and 2 Cooper's hawks yielded positive results for WNV nucleic acid via PCR assay.

Virus isolation—West Nile virus isolation was performed on aqueous humor samples from 7 red-tailed hawks and 1 Cooper's hawk (Table 1). The virus was isolated from the aqueous humor of 1 red-tailed hawk and 1 Cooper's hawk. Results of WNV isolation procedures involving tissue homogenates from 6 red-tailed hawks and 2 Cooper's hawks were negative.

Macroscopic and microscopic lesions—On gross dissection, mild intraocular hemorrhage was detected in the right eye of 1 bird. Linear white discoloration of the retina was evident in 1 bird (bird 1), and in birds 2 and 3, a landscape-like white discoloration of the retina was detected. In 1 of the latter 2 birds, the vitreous humor was mildly opaque. Posterior segment changes were not grossly visible after fixation in the remaining globes.

The degree and nature of the microscopic lesions in the red-tailed hawks and Cooper's hawks were similar. All

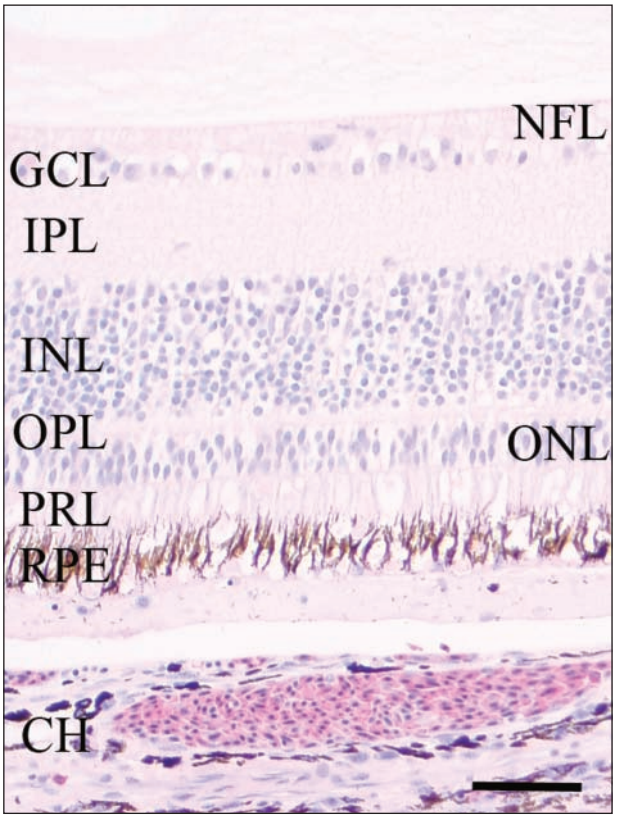

Figure 2—Photomicrograph of a section of an unaffected segment of choroid (CH) and retina illustrating the RPE cell layer, photoreceptor layer (PRL), outer nuclear layer (ONL), outer plexiform layer (OPL), inner nuclear layer (INL), inner plexiform layer (IPL), ganglion cell layer (GCL), and nerve fiber layer (NFL) of a red-tailed hawk. H&E stain; bar = 40 μm.

eyes with funduscopically visible lesions also had histologically detectable lesions. The normal avian retina is organized similarly to the mammalian retina and is comprised of 10 layers (**Figure 2**). In each examined bird, both eyes had microscopic lesions, although the degree of inflammation occasionally varied between them. The ocular lesions included disarray of the RPE cell layer, retinal necrosis, retinal atrophy, and lymphoplasmacytic and histiocytic uveitis (**Figures 3 and 4**). Pectenitis and papillitis were the most common forms of inflammation and were present in all birds that underwent histological examination (21 eyes [11 birds]; **Figure 5**). The degree of inflammation usually varied between the 2 eyes of each bird. Mild inflammation was present in 5 eyes (4 birds), moderate inflammation was present in 10 eyes (7 birds), and marked inflammation was present in 6 eyes (6 birds). Segmental multifocal choroiditis was identified in 14 eyes (11 birds), whereas mild iridocyclitis was present in 11 eyes (8 birds). The RPE cell layer overlying segments of choroid that were infiltrated by inflammatory cells was frequently in disarray. This disarray was evident as loss of the interdigitation of RPE cells and the photoreceptor cell layer as a result of loss of the processes of RPE cells. Frequently, there was also individualization and loss of RPE cells. The individualized RPE cells were plump and formed clusters; as a result, segmental clefts were present between the choroid and the overlying retina. Plasmacytic infiltration of

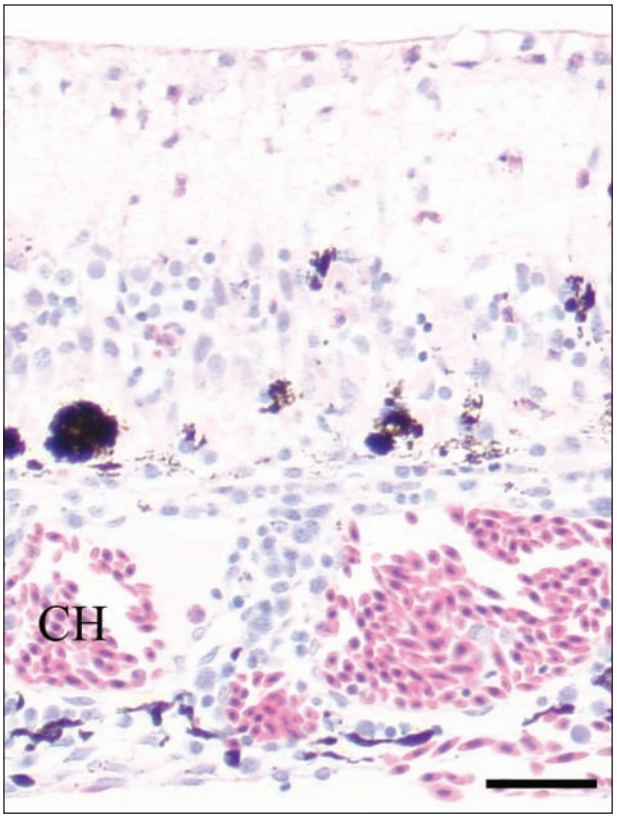

Figure 3—Photomicrograph of a section of the choroid (CH) and retina of a WNV-infected red-tailed hawk (bird 1). Notice the marked lymphoplasmacytic infiltration of the choroid and degeneration and necrosis of the RPE cells. The RPE cells have lost their typical interdigitation with the photoreceptor layer and are rounded up and clustered. The overlying retinal layers are disorganized with a loss of the distinction between outer and inner nuclear layers. H&E stain; bar = 40 μm.

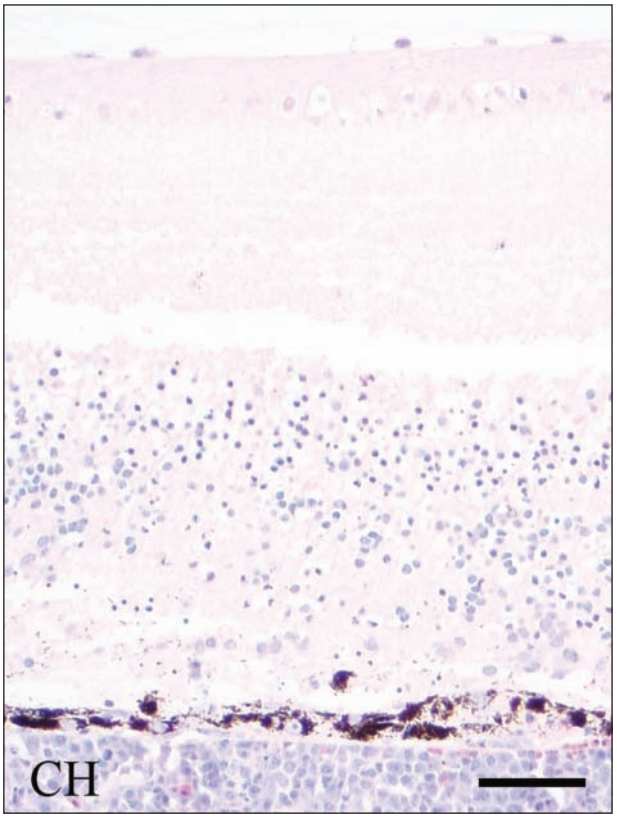

Figure 4—Photomicrograph of a section of the choroid (CH) and retina of a WNV-infected red-tailed hawk (bird 2). Notice the marked lymphoplasmacytic infiltration of the choroid and full-thickness necrosis of the retina. H&E stain; bar = 40 μm.

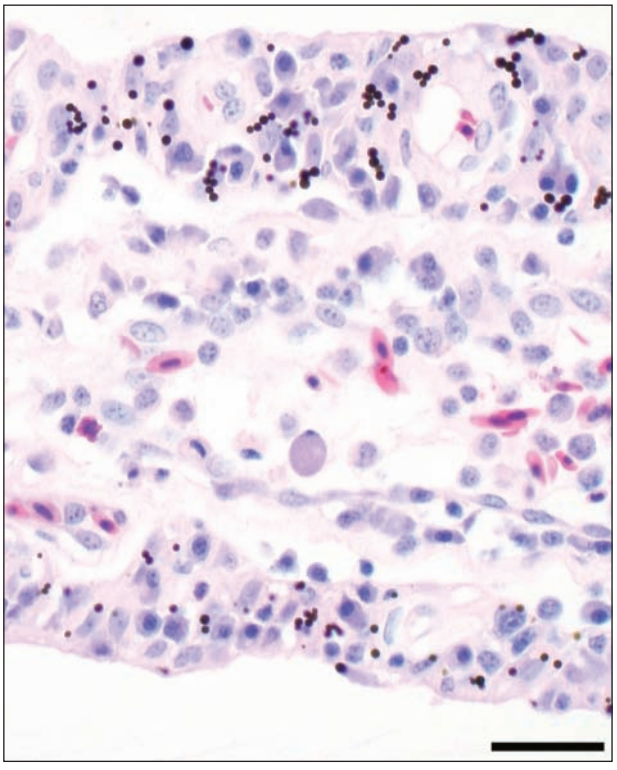

Figure 5—Photomicrograph of a section of the pecten of a WNV-infected red-tailed hawk (bird 6). Notice the scattered plasmacytic infiltration of the pecten. One leukocyte is parasitized by *Leukocytozoon* organisms. H&E stain; bar = 25 μm.

the nerve fiber layer of the retina was identified in 7 eyes (5 birds), whereas mild heterophilic retinitis that affected multiple layers was detected in 1 eye. The plasmacytic infiltration of the retina was usually most pronounced adjacent to the optic disc and gradually decreased toward the periphery. Disarray of the RPE cell layer was associated with necrosis of the inner and outer nuclear layers in 4 eyes (4 birds). Six eyes (4 birds) had segmental atrophy of the retina that occasionally resulted in a segmental loss of all layers of the retina. Full-thickness retinal necrosis was evident in 2 eyes (2 birds). In 1 eye, there was extensive ganglion cell necrosis. Homogenous eosinophilic material that resembled lens protein was present in the retina at the junction of atrophied and nonatrophied retina (retinal lenticular metaplasia) in 1 eye (bird 13).

Eight of the 11 birds had histiocytic and lymphoplasmacytic optic neuritis, which was bilateral in 3 birds. The remaining birds did not have evidence of optic neuritis in the examined nerve sections; however, in 1 of those birds, only the optic nerve of 1 eye was examined histologically.

Besides the ocular lesions, mild to moderate histiocytic and lymphoplasmacytic myocarditis was evident in 7 birds. All euthanized birds had a mild to marked encephalitis or meningoencephalitis with the exception of bird 13. The optic chiasm appeared to be the focus of inflammation in most birds, but lesions were fairly mild in the optic tectum.

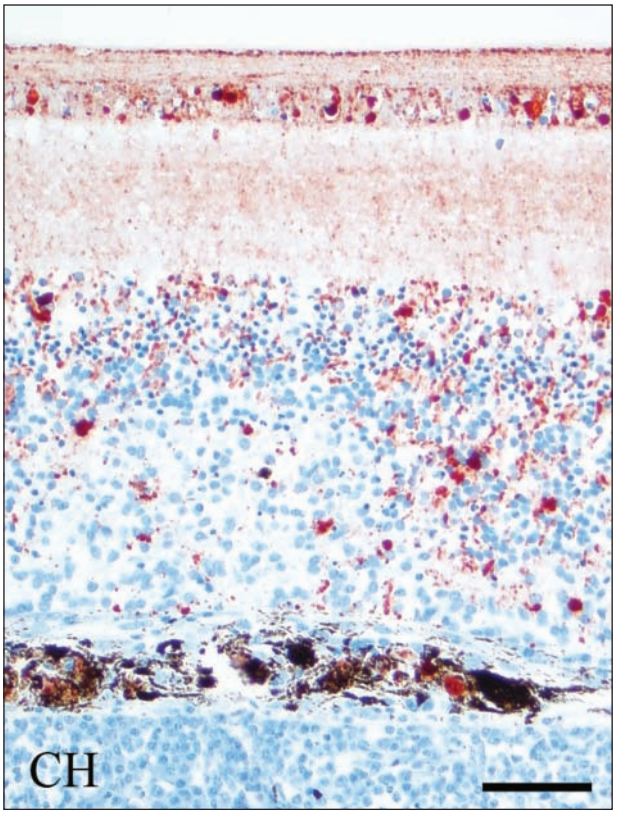

Figure 6—Photomicrograph of a section of the retina of a WNV-infected red-tailed hawk (bird 2) after immunohistochemical staining for WNV antigen. Notice that WNV antigen (reddish-brown stain) is evident in all retinal layers. The choroid (CH) is infiltrated by lymphocytes and plasma cells. Peroxidase-based polymer system involving anti-WNV antibody (clone 7H2); Mayer's hematoxylin counterstain; bar = 40 μm.

In the hawk eyes, WNV antigen was restricted to the retina. In 2 birds, the retina of both eyes contained WNV antigen-positive cells. In 7 birds, WNV immunoreactivity was detected in 1 eye only. In 2 of those birds, the immunoreactivity was considered marked (based on the number of WNV antigen–positive cells). The immunoreactivity in the retina was almost exclusively restricted to segments overlying choroid that were infiltrated by inflammatory cells. In 6 of the 9 birds with WNV immunoreactivity in the retina, the bodies of ganglion cells, the bodies of cells of the inner nuclear layer, and RPE cells were positive for WNV antigen (**Figure 6**). In addition, cellular processes in the nerve fiber layer and in the inner and outer plexiform layers were positive for WNV antigen. Occasionally (4 birds), there was strong staining of vertical cell processes in the inner nuclear layer (**Figure 7**). However, in 2 Cooper's hawks, a horizontal staining pattern was present at the level of the horizontal cells of the inner nuclear layer (birds 8 and 10). In 1 red-tailed hawk (bird 6) and 1 Cooper's hawk (bird 8), only few ganglion cells and the bodies of few cells in the inner nuclear layer were positive for WNV antigen. In 1 red-tailed hawk (bird 7), a weak granular stain was restricted to an area of retinal atrophy. Both eyes yielded negative results for WNV antigen in 2 red-tailed hawks (birds 5 and 13). In bird 5, there were only a few WNV antigen–positive smooth muscle cells

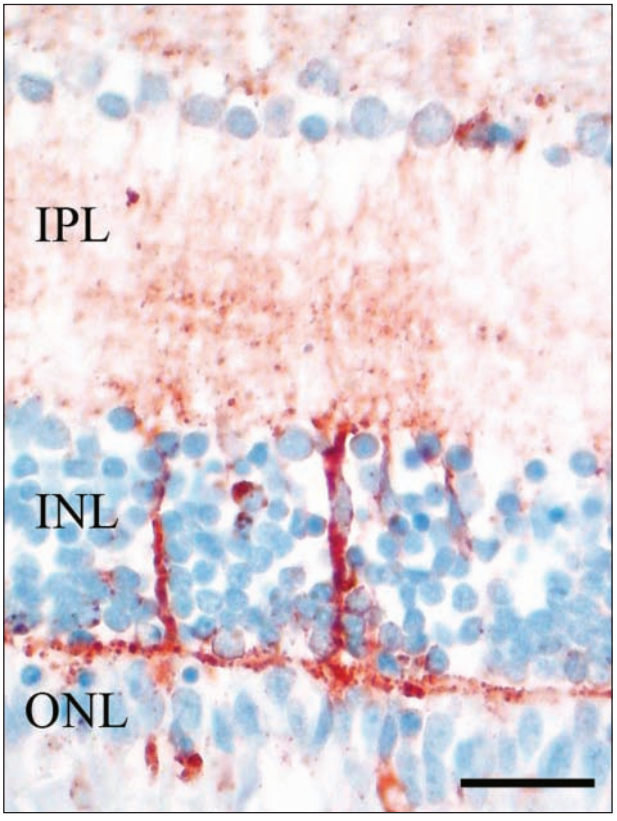

Figure 7—Photomicrograph of a section of the retina of a WNV-infected Cooper's hawk (bird 10) after immunohistochemical staining for WNV antigen. Notice that WNV antigen (reddish-brown stain) is evident in the outer nuclear layer (ONL), cell fibers of the outer plexiform layer, cells of the inner nuclear layer (INL) with vertical cell processes, cell processes in the inner plexiform layer (IPL), a ganglion cell, and cell processes of the nerve fiber layer. Peroxidase-based polymer system involving anti-WNV antibody (clone 7H2); Mayer's hematoxylin counterstain; bar = 25 μm.

in the tunica media of 1 myocardial artery. The hearts of the remaining 9 birds were negative for WNV antigen, whereas the heart of 1 bird was not examined. Few WNV antigen–positive tubular epithelial cells were identified in 3 birds, whereas the kidneys of the remaining 8 birds were negative for WNV antigen. For 1 hawk (bird 13), all organs examined were negative for WNV antigen.

Discussion

Infection with WNV results in largely nonspecific clinical signs in raptors including lethargy; weight loss; and, in some instances, vision loss or neurologic signs.[4,6,7] Although antemortem diagnosis of WNV infection in raptors is possible in some birds via a rapid antigen-capture assay of oropharyngeal and cloacal swabs, this test has relatively low sensitivity and specificity for detection of WNV antigen in diurnal raptors, compared with its sensitivity and specificity for use in northern owls, and the definitive diagnosis of WNV infection in raptors is typically made after death.[2]

In the present study, all red-tailed hawks and Cooper's hawks with WNV disease (confirmed by results of immunohistochemical examination and PCR assay of organ samples) that were examined ophthalmoscopically had fundic lesions, and in 9 of the 10 birds, the lesions were active and consisted of linear or geographic areas of chorioretinal exudates. Ophthalmic examination of hawks with neurologic signs may be a powerful tool with which birds with WNV disease can be differentiated from those that have other neurologic conditions. The presence of active fundic lesions consisting of geographic or linear areas of chorioretinal exudates may be supportive of a diagnosis of WNV disease in birds with neurologic disease that are submitted to rehabilitation clinics during mosquito season in regions in which the virus is endemic. The prevalence of chorioretinal lesions in hawks with WNV disease is unknown and may vary with the severity of the clinical disease. Hawks with mild WNV disease were not euthanized in the present study; thus, histologic and immunohistochemical findings in these birds are not known.

In the present study, WNV and SLE virus were the only infectious diseases specifically investigated, and other infections in the study birds cannot be completely ruled out. Aside from chorioretinitis associated with trauma, chorioretinal lesions have been attributed to systemic infection with *Salmonella* Typhimurium var Copenhagen, mycobacteria, paramyxovirus, and *Toxoplasma* sp.[17] The histologic lesions detected in the birds of our study were most consistent with a viral or possibly protozoal infection but not with a bacterial or fungal infection. Infectious microorganisms including protozoal cysts such as *Toxoplasma gondii*, bacteria, and fungi were not detected histologically in sections of tissues from any hawks in the present study.

Chorioretinal lesions in humans infected with WNV have been reported.[9,10,18] In a prospective study[12] of humans infected with WNV, 23 of 29 (79.3%) patients had multifocal chorioretinitis that was most often asymptomatic and self-limited. These lesions were often present in linear clusters that were oriented radially or arranged in a curvilinear pattern.[12] The linear arrays were suggested to follow the pattern of choroidal vessels.[18] In affected raptors, the cause of the linear pattern of arrangement of chorioretinal lesions remains uncertain. Many of the linear lesions in affected raptors in the present study did not track along the expected course of choroidal vessels, and may instead have tracked within the nerve fiber layer.

Serologic evaluation may aid antemortem diagnosis of WNV infection, and assessment of aqueous humor for anti-WNV antibodies may aid in the diagnosis of the disease after death when serum and plasma samples are no longer useful. However, interpretation of serologic findings requires careful consideration. Results of 1 study[19] indicated a seroprevalence of 9.2% among apparently healthy nestling red-tailed hawks, but data regarding the prevalence of antibodies against WNV in hawks that fledged and left the nest have not been published to our knowledge. The same study[19] reported a high seroprevalence (88%) in adult, apparently healthy, free-ranging Cooper's hawks. Similarly, 4 hawks in the present study did not have signs of WNV disease or ocular lesions but did have serologic evidence of WNV exposure. The antibodies detected in the aqueous humor may have resulted from local antibody production or may have leaked from the circulation into the aqueous humor, possibly because of a breakdown of the blood-aqueous barrier. Calculation of the Goldmann-Witmer coefficient would distinguish between leakage of antibodies and local antibody production in the aqueous humor, but this value could not be calculated in our study because total IgM or IgG concentrations in plasma and aqueous humor were not measured.

Results of virus isolation and PCR procedures for WNV performed on aqueous humor samples appear to be of limited value in the diagnosis of WNV infection in red-tailed hawks and Cooper's hawks. Approximately 50% of the samples from WNV-positive birds (confirmed by results of immunohistochemical and PCR evaluations of organ samples) in the present study yielded negative results for WNV nucleic acid via PCR assay of aqueous humor samples, and 75% yielded negative results via virus isolation of aqueous samples. Nevertheless, virus isolation was successful in 2 of the examined aqueous humor samples, but results were negative in all examined tissue homogenates. Although a general processing error of the tissue homogenates may be considered as a possible explanation, this observation is consistent with a finding of a recent experimental study[20] involving red-tailed hawks. In that study, the virus was more commonly isolated from the eye than any other site, including the brain, heart, and kidneys.

Ocular infection may develop hematogenously during the viremic phase of the disease or via extension from the CNS to the outer retina via the optic nerves. However, WNV antigen was not detected in the optic nerves from any of the raptors in the present study. Interestingly, WNV antigen was principally detected in retinal segments overlying choroid that contained an inflammatory infiltrate. The immune system appeared to recognize the retinal WNV infection, as evidenced by the presence of inflammatory infiltration of the choroid. The exact pathogenesis of WNV-induced ocular lesions is unknown, and it is likely a combination of

direct effects of the virus along with secondary effects associated with inflammatory response to the virus.

In the present study, 1 red-tailed hawk with an active chorioretinal lesion in 1 eye was euthanized; although the PCR and immunohistochemical evaluations yielded negative results for WNV nucleic acid and WNV antigen, respectively, this hawk had a high anti-WNV antibody titer in plasma and aqueous humor. It is possible that this hawk was infected with WNV, but that the affected regions within organs containing WNV antigen were overlooked during sectioning. Alternatively, WNV antigen and nucleic acid could have been eliminated by the time of euthanasia. However, it is also possible that the high anti-WNV antibody titer was not associated with an active infection but rather was attributable to prior exposure and that the active chorioretinal lesion was not associated with WNV infection.

Two anti–WNV antibody-positive red-tailed hawks with clinical signs of WNV disease, including poor body condition and ocular lesions, were rehabilitated and released. These hawks received supportive care including fluid therapy and administration of a nonsteroidal anti-inflammatory medication. To the best of our knowledge, these are the first reports of survival of hawks with clinically evident WNV disease.

The most important steps in establishing a diagnosis of WNV disease in most of the birds of the present study included the histologic and immunohistochemical evaluation of the pecten, retina, and choroid. Among the study hawks, pectenitis was the most consistent finding, and the retina was the most constant site of detectable WNV antigen. On the basis of the results of the immunohistochemical evaluation, RPE cells and neurons at all levels of the retina were the prime target for WNV in the eye. Histologically, the inflammation most severely affected the choroid, with corresponding outer retinal changes in many instances. Evidence of chorioretinal mineralization, which was suspected from the funduscopic appearance of some lesions, was not detected in the histologic sections from any of the raptors with confirmed WNV infection. The apparent regions of mineralization detected funduscopically in 5 of the WNV-infected birds in our study were small. Hence, it is possible that those areas were not evident in sections of the globes that were examined histologically.

On the basis of the findings of our study, it appears that ophthalmic and histologic examination of the eyes may be useful for substantiating a clinical or postmortem diagnosis of WNV infection in red-tailed hawks and Cooper's hawks, and also likely other falconiforme birds. In addition, clinical and postmortem investigation of the eyes of WNV-infected raptors may add insight into the pathogenesis of the disease, thereby increasing the understanding of WNV infection in humans. Fluorescein angiography, multifocal electroretinography, and electron microscopic investigation of the retina may help to further characterize the chorioretinal lesions in WNV-infected hawks.

a. Kowa SL-15 biomicroscope, Kowa Co Ltd, Torrance, Calif.
b. Keeler binocular indirect headset, Keeler Instruments Inc, Broomall, Pa.
c. Kowa RC-2 fundus camera, Kowa Co Ltd, Nagoya, Japan.
d. Envision-HRP, DAKO, Carpinteria, Calif.
e. Animal Health Diagnostic Laboratory, Cornell University, Ithaca, NY.
f. Minimum essential medium with Earl's salts, Gibco-Invitrogen, Grand Island, NY.
g. Ciprofloxacin hydrochloride, Bayer, Kankakee, Ill.
h. Guinea pig complement, Colorado Serum Co, Ft Collins, Colo.
i. Low–melting-point agarose, Invitrogen, Carlsbad, Calif.
j. Neutral red, Gibco-Invitrogen, Grand Island, NY.

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