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Amdoparvovirus-associated disease in striped skunks (*Mephitis mephitis*)

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Abstract

Disease caused by the archetypical amdoparvovirus (APV), Aleutian mink disease virus (AMDV), has been well studied, but APV infections in other carnivores are poorly understood. Skunk amdoparvovirus (SKAV), one of a handful of newly discovered APVs, is apparently species-specific in striped skunks (*Mephitis mephitis*) and has a high prevalence across North America. We have evaluated the infection status and viral tissue distribution in a cohort of 26 free-ranging California skunks from a single rehabilitation facility who were euthanized due to poor prognosis for recovery from neurologic disease. SKAV was detected in the majority of this cohort, and virus was associated with a spectrum of lesions including tubulointerstitial nephritis, meningoencephalitis, myocarditis, and arteritis. Affected tissue and patterns of inflammation were partially overlapping with those of AMDV infection but were notably distinct in the kidney.

Keywords

amdoparvovirus; histology; in situ hybridization; mephitis; Striped skunk

Amdoparvovirus (APV) is a rapidly growing genus within the viral family *Parvoviridae* comprising several important pathogens of carnivores.[6,10] The prototype and archetype Aleutian mink disease virus (AMDV, species *Carnivore amdoparvovirus 1*) has a high prevalence and causes significant losses of farmed mink, but no effective vaccine is available.[6,13] The fur industry has struggled for decades to eliminate AMDV. Farmed or experimentally infected mink can be clinically normal but, depending on viral strain and host genetics, infected animals can develop fatal disease, with common sequelae including chronic, slowly progressive dysregulation of the immune system and immune complex–mediated vasculitis and glomerulopathy.[9,15–17] APVs have been identified in many other

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carnivores including skunks, foxes, raccoon dogs, and red pandas, but clinical outcomes and genetic determinants of host tropism are not characterized.[2,4,12,18]

Our interpretation of the impact of any APV infection requires recognition of potential disease sequelae in the growing number of species known to harbor infections. Striped skunks (*Mephitis mephitis*) are ubiquitous in North America and share dens, food, and water sources with other animals, thereby serving as a reservoir of viruses that infect wildlife and domestic animals. Recent data demonstrates that this species harbors a phylogenetically distinct species called skunk amdoparvovirus (SKAV).[2,5] SKAV prevalence, estimated by detection in blood is remarkably high in all areas that have been tested (Canada, 86%, US 82%), but potential disease association, including the type and impact of disease, is unknown.[1,3,8,11] Most APVs, including SKAV, were discovered in the last decade, so disease association is further complicated by any literature prior to 2011 that could not distinguish among multiple potential viral species. Specific PCR detection for SKAV species has been performed in a few studies, but given that we have only recently uncovered the high prevalence of SKAV, any unchallenged declaration of disease association based on PCR alone is precarious.[2,5] Molecular detection of SKAV from diseased tissue coupled with viral distribution by in situ hybridization (ISH) both establishes disease association and reveals mechanisms of pathogenesis such as tissue targets of infection and pathways for SKAV shedding and transmission.

Previously documented SKAV tissue targets reported in 3 animals included cells of the kidney, gastrointestinal tract epithelium, and skin, with the conclusion that transmission was possible by any of these sources.[2] However, viral distribution in other tissues and direct association with lesions have not been evaluated. In this study, we used a retrospective cohort of 26 skunks and a combination of PCR and ISH to analyze the distribution of SKAV and its association with lesions in multiple tissues. All 26 cases were submitted for routine necropsy to one of two University of California laboratories (UC Davis Veterinary Medical Teaching Hospital [3 cases] or California Animal Health and Food Safety Laboratories [23 cases]). Cases were included in this study if complete necropsies were performed and adequate formalin-fixed, paraffin-embedded (FFPE) tissues were available. Three cases included in this study were also described in a previous report.[2] All animals (26/26) came directly from a single rehabilitation facility and were euthanized based on decisions by veterinary or rehabilitation center personnel that the animal had neurologic disease and a poor prognosis for recovery. This common clinical presentation was not an inclusion criterion per se, but rather a consequence of the reality that neurologic skunks are more likely to be captured and potentially harbor zoonotic disease (rabies). As a result, this tested cohort is heavily biased, both clinically and geographically.

The spectrum of neurologic signs from subtle (unafraid) to overt (obtunded) are listed in Supplemental Table S1. Antemortem trauma occurred in 1 case (vehicular trauma, spinal fracture). The cohort included 14 males, 10 females, and 2 skunks of undetermined sex. Seven skunks were juveniles, and 19 were adults. Rabies was ruled out in all cases by ancillary testing (19/26) or by histology alone (7/26). In addition, 16/26 skunks were tested by immunohistochemistry for the presence of canine distemper virus, and 15/16 skunks were negative. The cohort was variably tested for other pathogens, and trace or high levels of

anticoagulants (brodifacoum, bromethalin) were detected in 10 animals, including cases that were both SKAV positive and negative. Postmortem examination findings are summarized in Supplemental Table S1.

Splenic tissue is recognized as a sensitive sample for determining APV infection status.[6] Identification of SKAV-positive cases was based on PCR targeting a 365-nucleotide segment of the capsid gene from formalin-fixed, paraffin-embedded (FFPE) scrolls of splenic tissue or tissue pools (Supplemental Table S1). In six cases, PCR detection of Amdoparvovirus infection was performed by outside laboratories using assays designed to detect AMDV, but specific primer strategies were not disclosed by the laboratories. Selected primers in our laboratory, and those of the outside laboratories, could potentially amplify other APVs, but in a separate study, all amplicons sequenced (30/100) from skunk spleens were SKAV.[2] In this cohort, Amdoparvovirus infections (presumed SKAV) were detected in 20/26 (76.9%) skunks tested, including all four tested with assays for “AMDV.” This is similar to the prevalence previously reported in a collection of 101 skunks (82%), among which 23 were sourced from California.[2]

SKAV ISH was performed as previously described.[2] Based on tissue targeting of the related AMDV and the frequency of renal lesions in this cohort (21/26), we performed ISH on kidneys of all animals. Correlation of PCR amplification and ISH probe hybridization (in the kidney) was high. Of the 20 PCR-positive cases, 19 had detectable SKAV in the kidney. Of the 6 PCR-negative cases, 5 were ISH-negative in the kidney. The remaining PCR-negative case, for which FFPE spleen was not available, was PCR-negative in FFPE tissue pools containing kidney, GI tract, and lymph nodes, but nonetheless exhibited scant, multifocal positive probe hybridization in the kidney.

The predominant renal lesion in SKAV-infected skunks was a mononuclear interstitial infiltrate, often surrounding degenerate or necrotic tubules in the renal cortex with variable extension into the medulla (19/20 PCR-positive cases, 95%). The proportion of plasma cells in inflammatory infiltrates varied widely between cases. While similar inflammation is described in spontaneous Aleutian disease of mink, the “classical” observation of AMDV in the mink kidney is a glomerulopathy/glomerulonephritis, which was an uncommon finding in skunks (4/21 skunks with renal lesions; 19.1%).[9] In skunks, cortical and/or medullary segments of renal tubules were variably to widely separated by interstitial inflammation (Fig. 1). In affected areas, renal tubular epithelium exhibited one or more of the following changes: swelling with cytoplasmic microvacuolation (degeneration); cytoplasmic hypereosinophilia; or shrinking with nuclear pyknotic, karyorrhexis, and/or karyolysis (necrosis). Tubules were variably ectatic and contained hyaline, granular, or cellular casts. SKAV ISH probe hybridization was detectable in the kidneys of 19 cases. Viral nucleic acid was present in the tubular epithelium of cases that were minimally to severely affected, with probe hybridization present at all levels of the tubular nephron, as well as within glomerular parietal epithelium. Virus was detected in the most severely affected tubules in the section, including in necrotic, degenerate, remnant, or sloughed epithelial cells, suggesting a causative role in inducing tubular degeneration and necrosis. However, signal was also present in some tubules with minimal histologic changes, so it is additionally possible that renal tubular epithelium is a site of persistence. Viral nucleic acid

was also detected in infiltrating inflammatory cells—presumed macrophages—as well as in scattered individual cells morphologically resembling endothelial cells.

Additional histologic lesions identified frequently in this cohort included meningoencephalitis ($n = 17$), myocarditis ($n = 15$), and arteritis ($n = 8$). Because similar lesions have been associated with APV infections in other species, we tested representative cases by ISH to evaluate the possible association of SKAV in skunks.[2,7,17]

Meningitis, encephalitis, or meningoencephalitis were present in 17 PCR-positive skunks and 1 PCR-negative skunk. The lesions ranged from scant lymphoplasmacytic infiltrates to marked pleocellular inflammation and gliosis with regions of necrosis. In cases with meningitis, meningeal blood vessels were eccentrically or concentrically surrounded by mononuclear cells, and peripheral (penetrating) vessels within the neuropil were cuffed. Central nervous system (CNS) inflammatory lesions from two cases in this cohort were tested by ISH. Three cases that were excluded due to the unavailability of renal tissue sections were also tested. In 3/5 total cases tested, SKAV probe hybridized within scattered cells in the adventitia of affected vessels in the neuropil or meninges (Fig. 2a, b).

Myocarditis was observed in 15/20 PCR-positive skunks. This was primarily lymphoplasmacytic, multifocal, and in 5 cases was detected in combination with arteritis of the coronary arteries or their branches. Severe cases had dense infiltrates of plasma cells and lymphocytes disrupting the myocardium, admixed with neutrophils and macrophages and regions of necrosis that were attributed to infarction. Three cases were analyzed by ISH, and viral nucleic acid was detected in myocardial inflammatory infiltrates in 3/3 cases tested (Fig. 2c, d). Virus was not convincingly detected in cardiomyocytes.

Arteritis was present in 8 PCR-positive animals, most often involving muscular arteries including coronary arteries and their branches (5/8) or renal or arcuate arteries in the kidney (5/8). In affected arteries, the adventitia was concentrically to eccentrically surrounded by lymphocytes and fewer plasma cells that expanded and sometimes obscured the vasa vasorum. In segmental regions lymphocytes disrupted and separated layers of the muscular wall. Fibrinoid necrosis, characterized by smudgy, brightly eosinophilic arterial walls and intramural karyorrhectic cells, was present segmentally to circumferentially in three cases. In 3/3 cases tested by ISH, SKAV probe hybridization was detected in inflammatory cells surrounding and infiltrating affected arteries (Fig. 2e–h). Immunohistochemistry for α-smooth muscle actin (SMA) and factor VIII-related antigen were used to characterize arterial changes in select cases (Supplemental Material 1).

Splenitis has rarely been associated with APV infections in red pandas, and although spleen is the tissue of choice for diagnosis of infection in FFPE tissues by PCR, splenitis was only observed in 1 skunk in this study (Pesavento lab, unpublished). This finding suggests that the spleen provides a sensitive representation of viremia. This is further suggested by the distribution of SKAV in normal spleens, where viral nucleic acid was detected both in scattered round to polygonal cells—presumably circulating macrophages—in the red pulp and in a patchy distribution in germinal centers. The latter, by morphology, is presumed

to reflect infection of dendritic cells, similar to what has been proposed for red panda amdoparvovirus.[3]

In 2/3 available skin samples from PCR-positive cases, SKAV was detected in basilar cells of epidermal and follicular epithelium. In these cases, histopathologic findings were lymphoplasmacytic dermatitis underlying and occasionally extending into the epithelium, with apoptosis or necrosis of individual epithelial cells.

The archetype *Amdoparvovirus*, AMDV, is well-studied in the narrow genetic context of farm-raised mink, where the most common reason for renal failure is persistent infection leading to a hypergammopathy and immune-complex deposition within glomeruli.[6] In skunks, SKAV infection in a subset of infected skunks causes multisystemic disease that only partially overlaps with the related mink virus. The predominant lesion within the kidney of skunks was necrotizing tubulointerstitial nephritis, usually with regions acutely affected embedded in a background of fibrosis and nephron loss. The pattern suggests a multiphasic destruction that we propose is sequela of persistent SKAV infection. Meningitis, or meningoencephalitis, while common in this cohort, carries the important caveat that animals were submitted for necropsy because of neurologic disease. Alternate etiologies for central nervous system inflammation were not specifically identified in most cases; however, the demonstration of SKAV nucleic acid in inflammatory brain lesions supports the possibility of causality in at least a subset ogf cases. Sporadic findings like pneumonia, dermatitis, glossitis, enteritis, and hepatitis with associated virus demonstrate that SKAV can target multiple tissues.

Small carnivores are increasingly crowded in suburban settings. While most APV infections to-date appear to be species-specific, cross-species infections have been demonstrated in some cases.[4,14] SKAV was first recognized as distinct viral species in 2017, and since then has been demonstrated to have both a remarkably high prevalence—even among APVs—and remarkable genetic diversity—with up to ~15% sequence variation among individual isolates from infected skunks.[1,3,8] This genetic plasticity may have implications for virulence or host tropism, as it remains plausible that striped skunks could share infections with related, in-contact species.

Diagnostic recognition of the unique spectrum of both acute and chronic SKAV-associated lesions is prerequisite to estimating the potential impact of SKAV on the greater population of free-ranging skunks. SKAV infection should be a differential diagnosis for a variety of inflammatory lesions including nephritis, arteritis, meningoencephalitis, and myocarditis. Unique APV species have been identified from 5 carnivore species in the past 12 years, and among those studied, infection is highly prevalent and is associated with disease in a subset of animals.[2,4,5,12,18] AMDV has been detected in hosts other than mink, but we have no idea whether or how co-infections and/or host-switching influence clinical outcome. Comparisons of disease expression among hosts would benefit from pathogenesis studies that consider viral evolution and potential for viral spillover.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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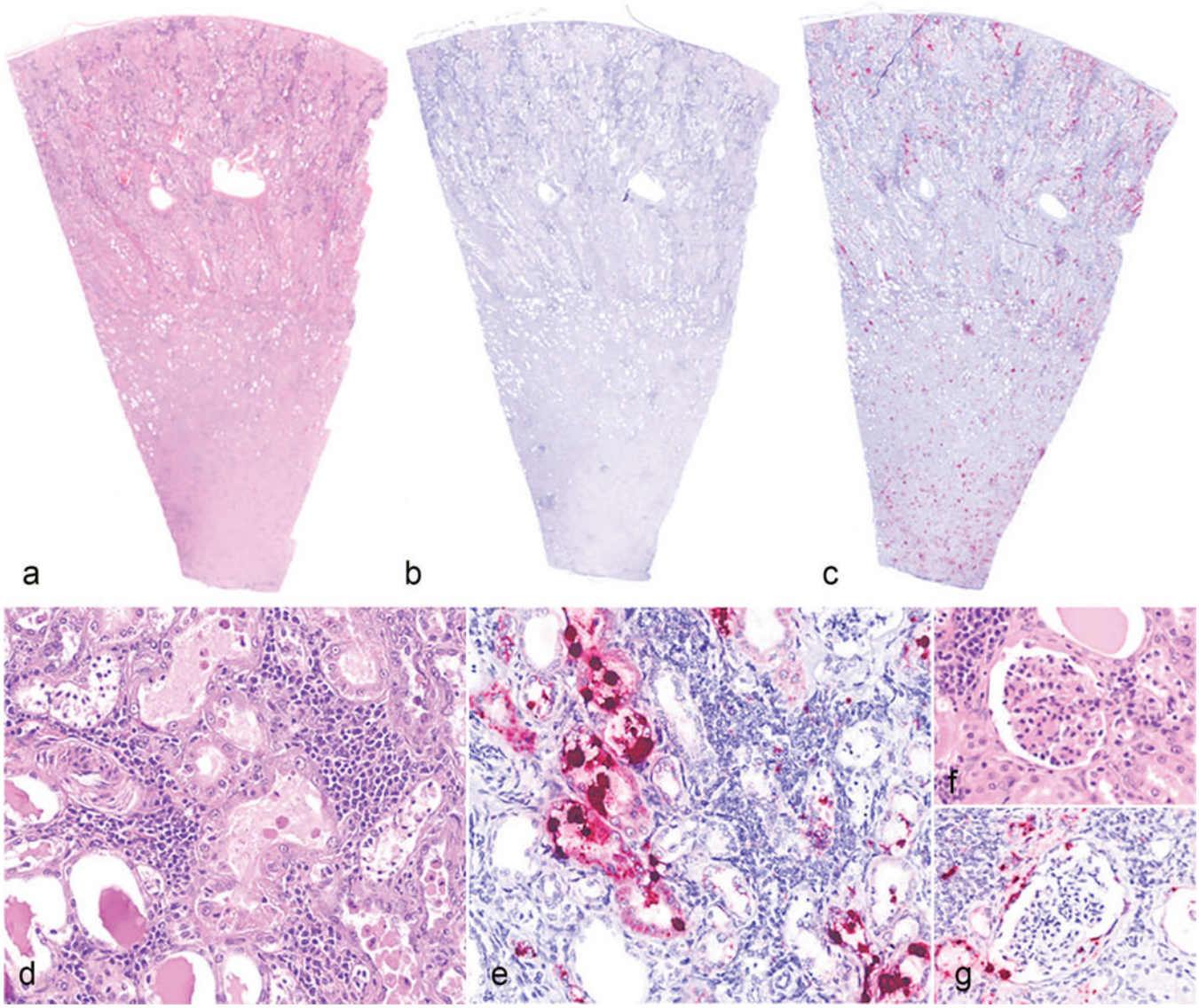

Figure 1.

Chronic tubulointerstitial nephritis, striped skunk, case 3. (a) Zones of dense hypercellularity separate or replace renal tubules.Hematoxylin and eosin (HE). (b) No hybridization observed with negative control (*DapB*) in situ hybridization (ISH) probe. (c) ISH with SKAV-specific probes demonstrates abundant viral nucleic acid (red) at all levels of the kidney. (d) Tubules are separated by dense aggregates of lymphocytes and plasma cells. Tubules are multifocally ectatic and often contain brightly eosinophilic luminal proteinaceous fluid (HE). (e) ISH signal is detected in renal tubule epithelial cells, in tubular protein casts, and scattered in inflammatory infiltrates. SKAV ISH. (f) Except where abutted by inflammatory lesions, glomeruli are essentially within normal limits. HE. (g) ISH signal is detectable in glomerular parietal epithelium without evidence of membranous glomerulopathy. SKAV ISH. SKAV, Skunk amdoparvovirus.

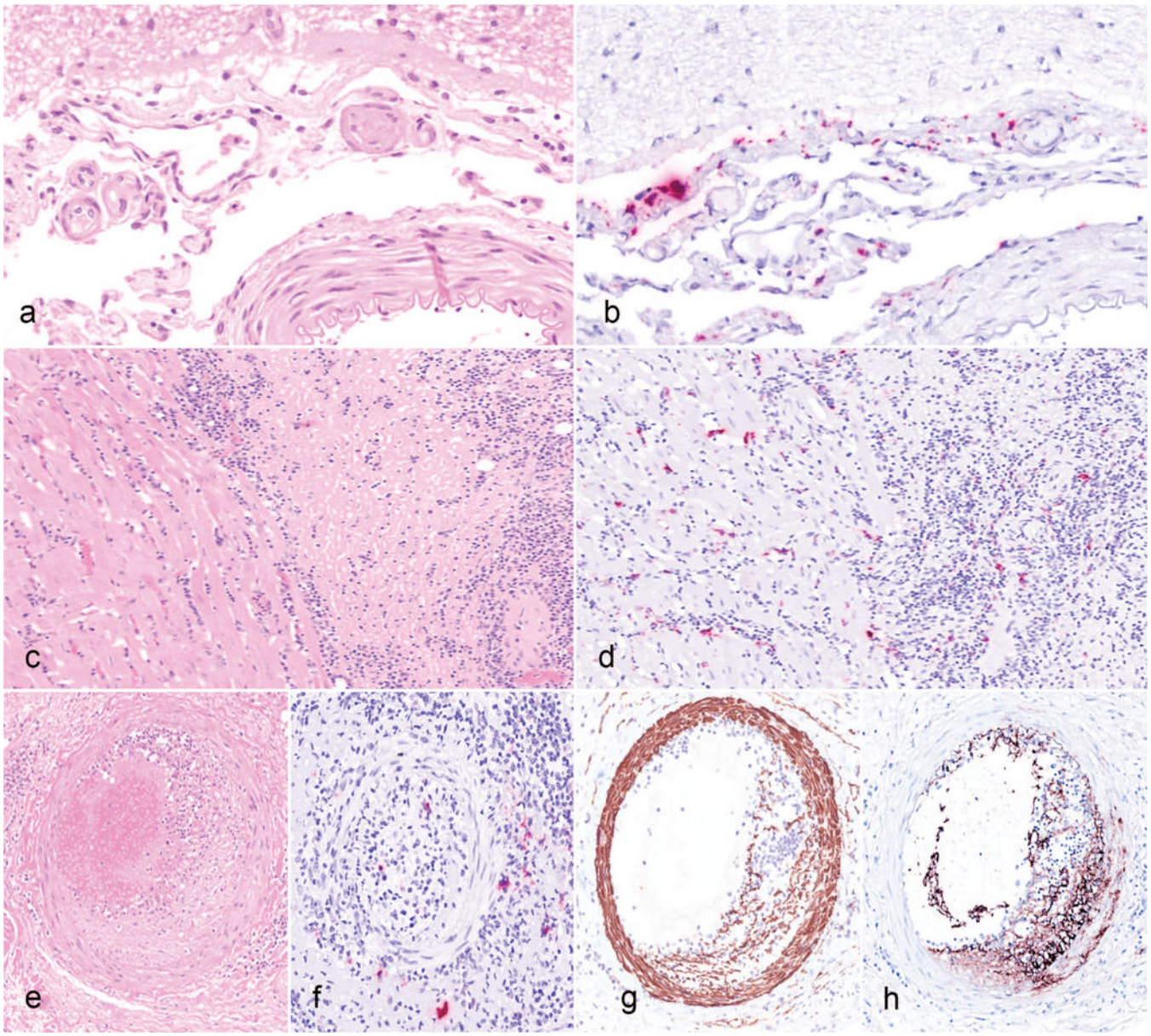

Figure 2.

Non-kidney targets of SKAV. (a–b) Meningitis, striped skunk, case 3. (a) Scant lymphocytic and plasmacytic inflammation is present in the meninges, particularly around small blood vessels. Hematoxylin and eosin (HE). (b) SKAV nucleic acid is detected in infiltrating inflammatory cells. SKAV in situ hybridization (ISH). (c–d) Myocarditis, striped skunk, case 17. (c) Lymphocytes and plasma cells multifocally expand the interstitium, admixed with fibrin. HE. (d) Viral nucleic acid is evident in inflamed regions, including in elongate cells between cardiomyocytes (suspect endothelial cells). SKAV ISH. (e-h) Arteritis, striped skunk, case 17. (e) Adventitial inflammation and segmental disruption of the muscular wall are evident, with infiltrating mixed inflammatory cells, fibrin, and eccentric expansion of the intima. HE. (f) SKAV nucleic acid is present amid periarterial and mural inflammatory cells. SKAV ISH. (g) Immunohistochemistry for smooth muscle actin (SMA) demonstrates partial

disruption of the muscular wall. (h) Immunohistochemistry for factor VIII-related antigen demonstrates immunoreactivity within the disrupted arterial wall, presumably indicating platelet extravasation due to loss of endothelial integrity. SKAV, Skunk amdoparvovirus.