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MOUNTAIN LIONS (*PUMA CONCOLOR*) RESIST LONG-TERM DIETARY EXPOSURE TO CHRONIC WASTING DISEASE

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ABSTRACT: For nearly 18 yr, we evaluated susceptibility of captive mountain lions (*Puma concolor*) to chronic wasting disease (CWD) in the face of repeated exposure associated with consuming infected cervid carcasses. Three mountain lions with a monomorphic prion protein gene (*PRNP*) sequence identical to that described previously for the species had access to parts of ≥432 infected carcasses during ≥2,013 feeding occasions, conservatively representing >14,000 kg of infected feed material, during May 2002 to March 2020. The proportion of diet in infected carcass material averaged 43% overall but differed from year to year (minimally 11–74%). Most infected carcasses were mule deer (*Odocoileus hemionus*; ~75%). We observed no clinical signs suggestive of progressive encephalopathy or other neurologic disease over the ~14.5–17.9 yr between first known exposure and eventual death. Histopathology revealed no spongiform changes or immunostaining suggestive of prion infection in multiple sections of nervous and lymphoid tissue. Similarly, none of 133 free-ranging mountain lion carcasses sampled opportunistically during 2004–20 showed immunostaining consistent with prion infection in sections of brainstem or lymph node. These findings align with prior work suggesting that CWD-associated prions face strong barriers to natural transmission among species outside the family Cervidae.

Key words: Chronic wasting disease, mountain lion, mule deer, *Odocoileus hemionus*, prion, puma, *Puma concolor*.

INTRODUCTION

Chronic wasting disease (CWD; Williams and Young 1980)—an infectious prion disease—naturally infects deer (*Odocoileus* spp.), wapiti (*Cervus canadensis*), and several closely related species in the family Cervidae. Over the past five or more decades, this disease has become established in free-ranging cervid populations in foci scattered across North America (US Geological Survey 2020). Unchecked CWD epidemics have the potential to destabilize affected cervid herds (Edmunds et al. 2016; DeVivo et al. 2017), thereby raising concerns for managing and conserving affected species as well as questions about broader ecological, socioeconomic, and perhaps health implications (Miller and Fischer 2016; Mysterud and Edmunds 2019).

Although the natural host range of CWD appears to be limited to cervids, several predatory species (including humans) may be exposed to prions by consuming infected carcasses (Stewart et al. 2012; Hannaoui et al. 2017). Mountain lions (*Puma concolor*) are the main nonhuman predator of mule deer (*Odocoileus hemionus*) in the foothills of northcentral Colorado and southeastern Wyoming where CWD has been present in the wild since at least the 1970s (Williams and Young 1992; Miller et al. 2000). Infected deer are relatively vulnerable to predation (Miller et al. 2008; Krumm et al. 2010; DeVivo et al. 2017); consequently, mountain lions probably have some degree of CWD exposure in areas where their ranges overlap endemic foci.

Here, we describe the results of a long-term study (nearly 18 yr) to evaluate natural susceptibility of captive mountain lions to CWD under conditions of prolonged, repeated exposure by consumption of infected deer and wapiti carcasses. Spongiform encephalopathy is not known in free-ranging mountain lions. However, we regarded experimental assessment of exposure and susceptibility important in clarifying the role that mountain lions might play in helping suppress CWD in cervid populations and, conversely, the potential ecological ramifications of susceptibility in an apex predator. When we conceived

TABLE 1. Individual dietary exposure and ultimate cause of demise for captive mountain lions (*Puma concolor*) receiving carcasses and meat from native cervids infected with chronic wasting disease, as summarized from available feeding records and assuming all three animals partook in each feeding opportunity. Longevity estimates based on an assumed birth date. See text and Supplementary Material for additional information.

Sex	Adult body mass (kg, mean±SD)	Dietary exposure			Longevity (yr)	Cause of demise
		Cumulative days[a]	Infected carcasses[b]	Infected feedings[b]		
Male	64.3±4.4	5,290	434	799	15.2	Chronic osteoarthritis
Male	71.2±7.9	6,528	498	869	18.5	Adenocarcinoma
Female	57.6±3.7	6,531	498	869	18.5	Chronic osteoarthritis

[a] Number of days between first known exposure to an infected carcass and death.

[b] The number offered for group feeding while this individual was part of the group. Minimum number based on available records. See text for details.

this study in 2001, the susceptibility of mountain lions (Willoughby et al. 1992), other wild felids (Kirkwood and Cunningham 1999, 2007), domestic cats (*Felis catus*; Hewicker-Trautwein and Bradley 2007), and humans (Will et al. 1996; Bruce et al. 1997) to bovine spongiform encephalopathy (BSE) prions underscored a broader potential value in understanding mountain lion susceptibility to CWD.

MATERIALS AND METHODS

Three orphaned, 4–6-wk-old mountain lions originating from near Chugwater, Wyoming, were acquired opportunistically from the Wyoming Game and Fish Department in October 2001. The native prion protein (*PRNP*) gene sequence for each subject animal was determined from extracts of genomic DNA by primers and methods described by Stewart et al. (2012; Supplementary Material).

The three siblings (one female, two males) resided together at the Foothills Wildlife Research Facility of the Colorado Division of Parks and Wildlife (Table 1). We held them in a fully enclosed ~224-m^2 pen for the first year, then in a larger, dividable ~275-m^2 covered outdoor pen connected to a building with three ~8-m^2 indoor cages that could be opened or closed to comingle or separate individuals. All three animals generally had access to the entire outdoor and indoor housing complex.

The two males were castrated and the female was spayed at about 5 mo of age to facilitate long-term tractability and to minimize potential for conspecific aggression. Routine veterinary care included regular use of vaccines against rabies (Imrab, Merial Inc., Duluth, Georgia, USA), feline leukemia (multiple products used), and a combination of feline panleukopenia, feline herpes, and feline calicivirus (multiple products used), as well as the anthelmintic praziquantel every 6–12 mo (Droncit Cestode Tabs, BayerDVM, Shawnee Mission, Kansas, USA).

Throughout their lives, all three mountain lions received a diet primarily composed of deer (*Odocoileus* spp., mainly mule deer) and wapiti carcasses or carcass parts, infrequently supplemented with moose (*Alces alces*), bighorn sheep (*Ovis canadensis*), or pronghorn antelope (*Antilocapra americana*). All three also probably caught and consumed small mammals and birds that on rare occasion strayed into their enclosure. Carcasses were prepared to retain the nervous system, lymphoid, and muscle tissues, and other organ tissues if available. Carcasses were whole, cut in half, or quartered. Initially the entire gastrointestinal tract was offered, but because gut tissue or offal generally were not consumed, we switched to offering mainly eviscerated carcasses with the heart, liver, and kidneys intact. We offered carcasses or parts daily when the study animals were young and growing, then every 3 or 4 d as they matured, to simulate natural feeding patterns, facilitate training, and combat obesity (Fig. 1).

Plans for this investigation were approved by the Colorado Division of Parks and Wildlife animal care and use committee (file 04-2002) in April 2002. The cubs first fed on a confirmed positive mule deer carcass on 3 May 2002. We assessed repeated exposure that would mimic patterns expected through a lifetime of natural foraging in a CWD-endemic area, with a goal that about 20% of the collective annual diet included CWD-positive tissues. Note that the highest observed prevalence in a free-ranging mule deer herd in Colorado in 2002 was about 15% among

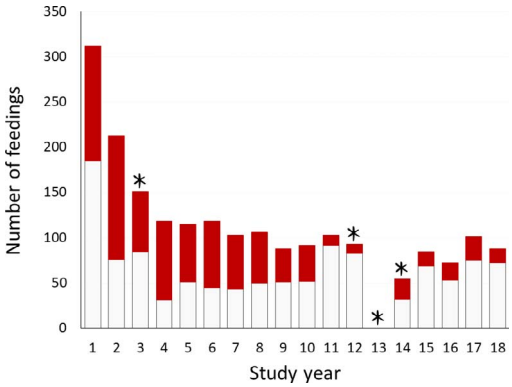

FIGURE 1. Temporal distribution of dietary exposure of three captive mountain lions (*Puma concolor*) to carcass materials from native cervids infected with chronic wasting disease (CWD). Bar segments show the cumulative number of CWD-positive (solid) and negative (open) feeding opportunities within successive 12-mo periods, as summarized from available feeding records and observed behavior assuming all three animals partook in each feeding opportunity. Study years defined as May–April (e.g., year 1 May 2002–April 2003). Asterisk (*) indicates study years that had ≥1 mo of missing feed records (late June to early August 2004; early March 2014 to late July 2015).

hunter-harvested adult males (Miller et al. 2000). We obtained deer and wapiti carcasses opportunistically from captive and free-ranging sources in conjunction with ongoing research, surveillance, and management programs, as well as meat donated by hunters who harvested test-positive deer and wapiti. Infected cervids represented all disease stages from early preclinical to end-stage clinical CWD. The distribution of positive or negative meat among feedings was irregular within and across years (Fig. 1) because most carcasses were fed to the lions before laboratory results were available.

All carcasses were tested by immunohistochemistry (IHC; Spraker et al. 2002a, b) or enzyme-linked immunosorbent assay (Hibler et al. 2003) of brain, lymphoid tissues, or both, primarily by the Colorado State University Veterinary Diagnostic Laboratory (Fort Collins, Colorado, USA). Group housing precluded determining individual consumption. Positive donated meat consumed during training and adjunct working activities (~0.5–5 kg/animal per session) augmented exposure. We estimated CWD exposure retrospectively and provide minimum estimates because we did not track the meat fed during training, and some records were missing or incomplete.

On the basis of our experience observing early signs of prion disease in cervids (Miller et al. 1998) and accounts of spongiform encephalopathy in nondomestic felids, including mountain lions (Willoughby et al. 1992; Kirkwood and Cunningham 1994, 1999), we recognized the clinical value of tractable and readily observed subject animals. Conventional veterinary neurologic examinations assess mentation, posture and gait, cranial nerve function, and postural reactions that may reveal deficits in coordination and strength. However, many of the necessary manipulations (e.g., "hopping," "wheel barrowing," "hemiwalking") to assess postural reactions are neither feasible nor advisable in captive mountain lions and other nondomestic animals. Consequently, we regularly conducted clinical assessments of subject animals to provide a sensitive indicator of infection, which is important from a scientific as well as an ethical standpoint. To facilitate these behavioral and other neurologic assessments, we trained all three mountain lions by positive reward-based operant conditioning (Skinner 1938; Pryor 1999; Westlund 2014; see Supplementary Materials for details). We selected trained behaviors that served as proxies for the elements of a conventional neurologic examination, essentially capturing and reinforcing natural behaviors as on-demand assessment tools by marking and rewarding them in the course of training and other interactions (Supplementary Material Table S1).

A veterinary pathologist (K.A.F.) examined each animal postmortem within 1–16 h of death and collected representative tissue samples (Supplementary Material). Distribution of spongiform lesions and disease-associated prion protein (PrP^d) accumulation in mule deer (Sigurdson et al. 1999, 2001; Fox et al. 2006) and felids (Ryder et al. 2001; Seelig et al. 2015) guided sampling and microscopic examinations (Supplementary Material). Subsamples were stored in 10% neutral buffered formalin, with paired samples frozen at –20 C. Fixed tissues were embedded in paraffin and sectioned at 5–6 μm, and multiple sections were examined by light microscopy after H&E staining (Supplementary Material). We referenced established criteria (e.g., Fraser and Dickinson 1968; Wells and Wells 1989; Wyatt et al. 1991; Willoughby et al. 1992; Williams and Young 1993; Wohlsein et al. 2012) in interpreting spongiform changes consistent with prion disease.

Multiple sections of brain, spinal cord, and tonsil; multiple lymph nodes; and other tissues were examined after IHC staining (Supplementary Material) for evidence of PrP^d accumulation, as per the Colorado State University Veterinary Diagnostic Laboratory protocol optimized for detecting PrP^d in cervids (Spraker et al. 2002a) with monoclonal antibody (mAb) F99/97.6.1 (O'Rourke et al. 2000). Additionally, we examined a subset of tissue sections by a second IHC protocol (Seelig et al. 2015) optimized for detecting PrP^d in cats experimentally infected

with CWD (Mathiason et al. 2013). This subset included caudal brainstem (dorsal motor nucleus of the vagus nerve and solitary tract nucleus) and sites consistently reported as showing PrP^d accumulations in prion-infected felids and cervids (Spraker et al. 2002b; Hilbe et al. 2009; Seelig et al. 2015). The Seelig et al. (2015) optimized protocol with mAb L42 (Vorberg et al. 1999) was run by CSU's Prion Research Center (Fort Collins, Colorado, USA); caudal brainstem sections from experimentally infected and uninfected cats (Mathiason et al. 2013) were used as IHC controls. We referenced multiple publications in establishing criteria for interpreting IHC staining patterns, including work by Bell et al. (1997), Ryder et al. (2001), Gill et al. (2013, 2020), Seelig et al. (2015), and Williams et al. (2018). See Supplementary Material for further details and additional references.

To complement experimental findings, we summarized IHC results for brainstem (medulla oblongata sectioned at the obex) and retropharyngeal lymph node tissues collected opportunistically from 133 free-ranging mountain lion carcasses originating from various locations in Colorado and screened for evidence of prion infection during 2004–20 according to the Colorado State University Veterinary Diagnostic Laboratory protocol.

RESULTS

Weekly neurologic assessments by operant conditioning revealed no clinical signs suggestive of progressive encephalopathy or other neurologic disease in the mountain lions. All three study animals maintained their unique individual personality and behavioral traits throughout their respective lifetimes and showed continued tractability and willingness to train and work (Fig. 2). Because of chronic osteoarthritis, we euthanatized one male at ~15.2 yr and the female at ~18.5 yr (5,290 and 6,531 d) after first known exposure; the other male unexpectedly died (metastatic cholangiocarcinoma) at ~18.5 yr (6,528 d) after first known exposure (Table 1 and Supplementary Material).

All three of the captive mountain lions matched the monomorphic *PRNP* sequence for this species reported by Stewart et al. (2012). Thus, despite being related and limited in number, we regarded these subject animals as broadly representative of mountain lions as a species with respect to their *PRNP* sequences.

Histopathology results indicated an absence of spongiform changes (neuronal vacuolation or neuropil spongiosis) typical of the central nervous system lesions seen in prion diseases (Fig. 2). Moreover, no immunostaining suggestive of prion infection was observed under either IHC protocol (Fig. 2). The full reports are included as Supplementary Material. In a few instances nonspecific staining was noted, including staining of atypical structures (e.g., staining of parasitic sarcocysts with mAb F99/97.6.1; inconsistent staining of vessel walls with mAb L42), as well as background staining (e.g., diffuse, wispy, or globular deposits of stain not specific in color or shape for PrP^d deposits). None of the 133 free-ranging mountain lions, including at least 86 adults (≥2 yr old) and 31 subadults (1–2 yr old), showed immunostaining consistent with prion infection.

On the basis of 2,013 feeding records, we estimated that all three mountain lions had access to parts of at least 432 infected carcasses (Table 1); more than 500 CWD-positive cervids were offered to the two longer lived animals. The majority of infected carcasses (n=492) were mule deer (~75%), with wapiti (~18%) and white-tailed deer (*Odocoileus virginianus*; ~7%) comprising the balance. Conservatively this represented >14,000 kg of infected carcass material offered, although only a portion was consumed. The proportion of infected carcass material averaged 43% over all 18 yr, but differed considerably from year to year (11–74%; Fig. 1). Exposure was underestimated—especially in years 11–18—because the supplemental positive meat fed in the course of training was not included in the annual total.

DISCUSSION

Our findings align with a prior assessment (Stewart et al. 2012) suggesting that mountain lions appear relatively unlikely to acquire CWD through natural dietary exposure. Despite hundreds of opportunities for prion exposure that simulated predation on infected

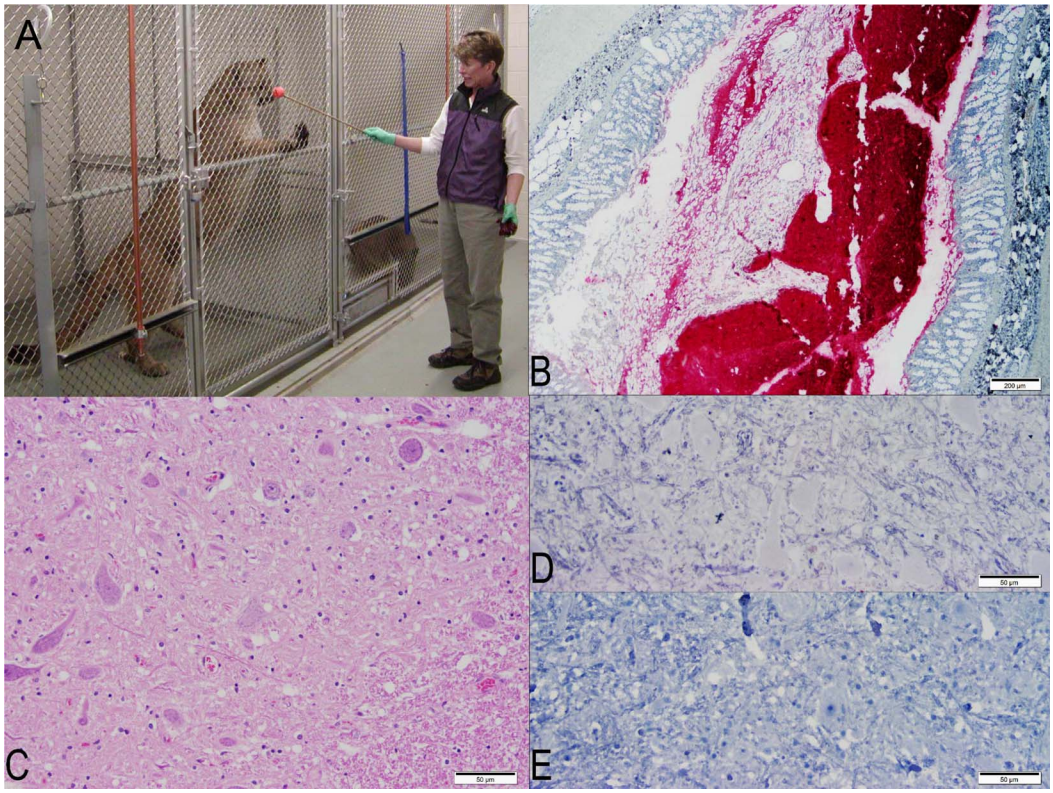

FIGURE 2. Antemortem and postmortem evaluations revealed no evidence of prion infection in three captive mountain lions (*Puma concolor*) consuming chronic wasting disease–infected carcasses for ≥14.5 yr. (A) Neurologic assessments by operant conditioning (Supplementary Material Table S1) revealed no clinical signs suggestive of progressive encephalopathy or other neurologic disease. (See Supplementary Video S2 of representative assessments.) (B) Intense immunohistochemistry (IHC) stain in cecal contents but not in gut-associated lymphoid tissue. Bar=200 µm; IHC with monoclonal antibody (mAb) F99/97.6.1 (slide 12, report WHL 16 949 in Supplementary Material). (C–E) A few areas of mild vacuolation (C) were observed (pictured: external cuneate nucleus of the brainstem; H&E stain), but no immunostained PrP^d accumulations after IHC staining with either mAb F99/97.6.1 (D) or L42 (E). Bar=50 µm in panels C–E, all from the same section of external cuneate nucleus (slide 7, WHL 16 949).

cervids, and nearly two decades for potential incubation, neither clinical nor postmortem assessments indicated the mountain lions under study contracted prion disease. The amount of infected carcass material was designed to exceed exposure of mountain lions preying in endemic mule deer ranges in Colorado or Wyoming circa 2002 (Miller et al. 2000). Experimental exposure began at a relatively early age. Over the first decade, on average more than half of the carcasses fed were infected (mainly adult cervids including at least 200 carcasses in later stages of clinical CWD). By comparison, although prey killed by mountain lions studied along Colorado's northern Front Range included a high proportion of mule deer (~64%; Blecha and Alldredge 2017), deer in the adult age classes most likely to be infected with CWD accounted for only ~30% of the prey taken.

Because the animals under study were confined and fed regularly, their consumption of carcass material seemed less catholic than in free-ranging counterparts. Nonetheless, carcass offerings and feeding patterns approximated those associated with presumptive BSE cases in mountain lions and other felids in zoos (Willoughby et al. 1992; Kirkwood and Cunningham 1994, 2007).

Our study maximized the potential for dietary exposure in the captive mountain lions. Skeletal muscle tissues from CWD-infected mule deer harbor infectious prions (Angers et al. 2006), and PrP^d occurs widely in lymph nodes and other organs (Sigurdson et al. 2001; Fox et al. 2006). Repeated exposure increases the probability of infection from low prion doses (Diringer et al. 1994, 1998) and probably drives transmission among natural host species (Miller et al. 2004; Tamgüney et al. 2009). Moreover, infectivity in lymphatic tissues can be high: Wolfe et al. (2012) precipitated rapid onset of clinical CWD in 17 mule deer exposed by oral inoculation with only ~0.1 g of tonsil tissue.

Limited screening of free-ranging mountain lions from CWD enzootic areas revealed no evidence of prion infection, reinforcing our experimental findings. Mountain lions have become increasingly abundant in Colorado since the mid-1960s (Apker and Vieira 2017), even as CWD emerged throughout much of the state. Although the indirect effects of CWD on mountain lion abundance by the effects on abundance or vulnerability of their main prey remain a possibility, human intolerance presents a more proximate limit on mountain lion abundance and survival throughout most of their ranges overlapping CWD occurrence (Apker 2017; Apker and Vieira 2017). On the basis of field estimates of annual survival (Moss et al. 2016), our study animals lived well beyond the life expectancy of free-ranging mountain lions in northcentral Colorado (~1.8–9.5 yr depending on sex and habitat). The advanced ages of our study animals also exceeded the reported lifespans of BSE-infected captive wild felids (Kirkwood and Cunningham 1999, 2007). Three mountain lions that contracted BSE in UK zoos developed clinical disease at ~5–12 yr of age, although true ages and timing of exposure were uncertain for the two longer lived individuals (Kirkwood and Cunningham 1999).

Our investment in training and regular working interactions with the three exposed animals facilitated a sustained clinical assessment that was highly sensitive to detecting early signs consistent with prion disease (Miller et al. 1998). Ataxia, sometimes accompanied by tremors, was seen most often in mountain lions and other felids affected by BSE (Kirkwood and Cunningham 2007). Early signs, including apparent lameness and behavioral changes, were more variable and subtle and thus difficult to discern in fractious individuals. The index feline spongiform encephalopathy–affected mountain lion presented with ataxia, hypermetria, and falling accompanied by "unusual" behaviors reported by keepers (Willoughby et al. 1992). Behavioral changes (especially aggression or timidity), tactile and auditory hyperesthesia, polyphagia, and altered grooming habits have been noted more frequently than locomotor dysfunction among BSE cases in domestic cats (Leggett et al. 1990; Wyatt et al. 1990, 1991; Hewicker-Trautwein and Bradley 2007). Similarly, subtle clinical signs, including stilted gait, weight loss, anorexia, polydipsia, patterned motor behaviors, head and tail tremors, and ataxia, occurred in cats infected with CWD after intracerebral injection (Mathiason et al. 2013). Despite the use of a clinical approach designed to detect even subtle signs of encephalopathy, none of the three animals we studied showed altered personality or behavior or any of the more striking neurologic signs described in transmissible spongiform encephalopathy–affected felids. Beyond the immediate health monitoring and tangible animal welfare benefits (Westlund 2014), the extent of their training and willingness to work also allowed all three animals to be further trained and used in complementary studies over time, such as testing collars, trap designs, biomarkers, noninvasive sampling methods, and even metabolic physiology measures (Williams et al. 2014; see Supplementary Material Video S1).

Domestic cats orally challenged with brain tissue from a CWD-infected mule deer also failed to contract prion disease over a 1.5–7-yr (19–85-mo) observation period (Mathiason et al. 2013) despite some susceptibility to intracerebral challenge. Regardless of the practical value in predicting the outcome of

exposure to prions by natural routes, the lesion profiles described in cats successfully infected with intracerebral exposure on first passage provided reference data for the postmortem screening undertaken here. A comparative summary by Seelig et al. (2015; see their table 4) of PrP^d distribution in CWD- and BSE-infected cats provided a framework for assuring that the examinations of central nervous system tissues from experimental and free-ranging mountain lions were sufficient to detect evidence of prion disease if present.

Nonspecific IHC staining observed in CWD-exposed mountain lions resembled descriptions of BSE in cats and humans (Bell et al. 1997; Hilbe et al. 2009; Gill et al. 2013, 2020). Some areas of mild vacuolation were noted in one male, but in the absence of clinical signs and immunostained PrP^d accumulations in central nervous system sections, they were attributed to autolysis (longest postmortem interval), age, or euthanasia (Wells and Wells 1989; Gould et al. 2003; Wohlsein et al. 2012; Williams et al. 2018). Similar incidental lesions have been seen in aged cattle exposed to CWD (Gould et al. 2003; Williams et al. 2018). None of the mountain lion lymphoid tissues had PrP^d accumulations. Lymphoid involvement is prominent in CWD cases among cervids (Williams 2005; Fox et al. 2006) and in humans infected with the BSE agent (Ironside et al. 2000). However, BSE-infected felids show inconsistent PrP^d accumulation in lymphoid tissues (Ryder et al. 2001; Hilbe et al. 2009; Eiden et al. 2010), and lymphoid PrP^d was not reported among CWD-infected cats.

An extensive genetic assessment of potential susceptibility to prion diseases in carnivore species revealed that mountain lion samples (n=100, all from Colorado) lacked *PRNP* gene sequence variation (Stewart et al. 2012), showing a monomorphic sequence also shared by our three subject animals. Moreover, mountain lions and feline spongiform encephalopathy–infected cheetah (*Acinonyx jubatus*) share a common PrP amino acid sequence (Stewart et al. 2012). Both species suffered disproportionately high numbers of BSE cases in zoo collections (Kirkwood and Cunningham 1994, 1999). Wild type mountain lions appear less susceptible to CWD-associated prions currently in Colorado than to BSE-associated prions, perhaps a further reflection of the strain differences between BSE and North American CWD prions (Bruce et al. 1997; Barria et al. 2014). Collectively, the findings described here and in similar long-term studies with various species (e.g., Rhyan et al. 2011; Williams et al. 2018; Fox et al. 2021) help to define the limits of the natural host range of CWD and identify apparent barriers to interspecies transmission.

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SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/JWD-D-21-00029.

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