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Avian trichomonosis in spotted owls (*Strix occidentalis*): Indication of opportunistic spillover from prey

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ABSTRACT

Avian trichomonosis, caused by the flagellated protozoan parasite *Trichomonas gallinae*, has variable pathogenicity among bird species ranging from asymptomatic infections to severe disease periodically manifesting in epidemic mortality. Traditionally, columbids are identified as highly susceptible to infection with occasional spillover into raptors that prey on infected birds. We identified avian trichomonosis in two dead California spotted owls (*Strix occidentalis occidentalis*) and three dead northern spotted owls (*S. o. caurina*) in California during 2011–2015; infection was confirmed in four owls by PCR. Pathologic lesions associated with trichomonosis in the owls included caseonecrotic lesions of the upper palate accompanied by oropharyngitis, cellulitis, myositis, and/or sinusitis. Spotted owls are known to mainly feed on small mammals; therefore, the source of infection as well as the significance of the disease in spotted owls is unclear. These owl trichomonosis cases coincided temporally and spatially with three trichomonosis epidemics in band-tailed pigeons (*Patagioenas fasciata monilis*). The same parasite, *T. gallinae* subtype A2, was isolated from the spotted owls and band-tailed pigeons, suggesting the owls became infected when opportunistically feeding on pigeons during mortality events. Avian trichomonosis is an important factor in the decline of the Pacific Coast band-tailed pigeon population with near-annual mortality events during the last 10 years and could have conservation implications for raptor species at risk, particularly those that are facing multiple threats.

1. Introduction

Avian trichomonosis is a disease caused by a protozoan parasite, most commonly *Trichomonas gallinae*. Avian trichomonosis has been reported most frequently in pigeons and doves (Stabler and Herman, 1951); however, infections recently have been documented in several species of songbirds (Anderson et al., 2009; Forzan et al., 2010; Robinson et al., 2010). The emergence of avian trichomonosis in these species has invigorated work to advance understanding of parasite diversity, pathogenicity, and host range. Genetic typing of the protozoan parasites is accomplished using PCR, identifying 15 different internal transcribed spacer (ITS) subtypes of *T. gallinae* isolated from a diversity of avian species, with some subtypes more commonly found in certain species (Gerhold et al., 2008; Grabensteiner et al., 2010; Sansano-Maestre et al., 2009). These subtypes have been further classified by sequencing the hydrogenosomal Fe-hydrogenase gene, of which two predominant subtypes exist, delineated A1 and A2 (Girard et al., 2014b; Lawson et al., 2011). Generally, subtype A1 is isolated from songbirds, columbids, and raptors in Europe while A2 is less commonly isolated and was recently found to be the predominant subtype isolated from Pacific Coast band-tailed pigeons (*Patagioenas fasciata monolis*) in California, USA (Girard et al., 2014b). Genetic evaluation of the parasite gives us insight into the ecology and epidemiology of avian trichomonosis and refines our understanding of host associations, cross-species transmission, and spillover events.

Trophic transmission of *T. gallinae* has been documented in raptors that feed heavily on infected prey such as mourning doves (*Zenaida macroura*) and rock pigeons (*Columba livia*) in mostly suburban and urban environments. Nestlings may then become infected with trichomonads directly from infected parents or indirectly when parents feed their young infected prey (Forrester

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and Foster, 2008). For example, avian trichomonosis was the cause of death for approximately 80% of Cooper's hawk (*Accipiter cooperii*) nestlings in urban locations in Arizona while none of the nestlings in rural locations died of avian trichomonosis; although few dead nestlings were detected in rural areas (Boal, 1997). The diet of urban Cooper's hawk pairs was comprised of 83% doves versus 10% for rural pairs. In northeastern Germany, 65% of northern goshawk (*A. gentilis*) nestlings were positive for trichomonads (Krone et al., 2005) while 39% of northern goshawk nestling mortality was attributed to avian trichomonosis in Great Britain (Cooper and Petty, 1988). In both cases, a large portion of the nestlings' diet included rock pigeons and wood pigeons (*C. palumbus*). In Spain, approximately 36% of endangered Bonelli's eagle (*Hieraaetus fasciatus*) nestlings were infected with trichomonads while avian trichomonosis accounted for 22% of total nestling mortality; the nestlings' diet consisted of rock pigeons and wood pigeons (Real et al., 2000). These findings suggest raptors inhabiting urban and suburban environments are more prone to infection. However, avian trichomonosis affecting raptors in rural and remote areas is more likely to go undetected.

An equally important, but often overlooked, source of infection in raptors may occur during avian trichomonosis epidemics, or mortality events, in prey species in which there may be hundreds to thousands of birds dying of the disease in a localized geographic area. In California, avian trichomonosis mortality events have been documented in mourning doves and band-tailed pigeons (Cole, 1999; Girard et al., 2014b; Rogers et al., 2016; Stromberg et al., 2008). Events in mourning doves typically occur during the spring and summer and are frequently associated with artificial feeding sources in urban and suburban areas (Stabler and Herman, 1951) which may result in infection in both adult raptors and their young when feeding on infected doves in these areas. Events in band-tailed pigeons occur most commonly in winter when almost the entire population of Pacific Coast band-tailed pigeons is overwintering from central to southern California, forming large flocks enabling the disease to more easily spread between individual birds at communal watering sites in mostly rural areas (Rogers et al., 2016). Avian trichomonosis mortality events have been documented in band-tailed pigeons since the mid-1940s and are an important factor in their decline (Rogers et al., 2016). Band-tailed pigeons inhabit oak woodland and coniferous forests of the Coastal and Sierra Nevada mountain ranges (Keppie and Braun, 2000); therefore, trichomonosis outbreaks in band-tailed pigeons may provide an opportunistic source of prey and subsequent infection among raptor species in these habitats.

Given their range overlap with band-tailed pigeons, spotted owls (*Strix occidentalis*) are at risk of exposure to *T. gallinae* during trichomonosis epidemics. Northern spotted owls (*S. o. caurina*) were listed as threatened under the Endangered Species Act in 1990 and are currently under review for listing in California. In California, northern spotted owls occur primarily in the Klamath and Coastal mountain ranges from Del Norte to Marin counties (Gutierrez et al., 1995). California spotted owls (*S. o. occidentalis*) were identified as a species of special concern in California in 2008 (Davis and Gould, 2008) and are currently under status review by the U.S. Fish and Wildlife Service (Federal Register Vol. 80, No. 181). California spotted owls inhabit coniferous forests of the Sierra Nevada and Tehachapi mountain ranges and the southern extent of the Coastal Mountain Range from Monterey to Santa Barbara counties (Gutierrez et al., 1995). Spotted owls are habitat specialists whose populations have declined primarily due to loss and alteration of preferred habitat. Spotted owls are non-migratory although individuals may make seasonal elevational movements in response to weather and prey availability (Gutierrez et al., 1995). Small mammals including leporids, squirrels, voles, gophers, and rodents make up the majority of the diet of spotted owls with dusky-footed woodrats (*Neotoma fuscipes*) and northern flying squirrels (*Glaucomys sabrinus*) being important to both northern and California spotted owls (Smith et al., 1999; Thrailkill and Bias, 1989; Ward et al., 1998). During an avian trichomonosis mortality event, slow-moving, sick or dying birds are increasingly available to predators such as owls that, through prey consumption, can become exposed to *T. gallinae*. Poor detection of sick and dead owls in more remote habitats makes this risk difficult to assess. We hypothesized that avian trichomonosis may be a locally important source of mortality in owls, particularly during epidemics in band-tailed pigeons.

In this study, we identify spillover infections in a sensitive raptor species coinciding with mortality events in band-tailed pigeons detected in California between 2011 and 2015. We describe the epidemiology of trichomonosis in spotted owls examined post-mortem and genetically identify the infecting trichomonads.

2. Materials and methods

The California Department of Fish and Wildlife (CDFW) Wildlife Investigations Laboratory (WIL) investigates causes of mortality in the state's wildlife. Incidents of sick and dead wildlife are reported to WIL by CDFW regional staff, other agencies, wildlife rehabilitation centers, and the public. When available, carcasses are collected and submitted to WIL (Rancho Cordova, CA) or the California Animal Health and Food Safety Laboratory (CAHFS; Davis, CA) for post-mortem examination.

2.1. Band-tailed pigeon investigations

Avian trichomonosis mortality events (defined as ≥5 birds dying in the same geographic location over several days to weeks) occurring in band-tailed pigeons in California between 1945 and 2014 have recently been summarized by Rogers et al. (2016). During mortality events between 2011 and 2015, a subset of carcasses was collected from outbreak locations for post-mortem examination and sampling for trichomonad isolation. A cotton-tipped applicator moistened with sterile saline was used to swab the oral cavity of recently dead pigeons as previously described (Girard et al., 2014a). The swabs were placed into InPouch™ TF culture devices (BioMed Diagnostics, White City, OR) and kept at 25 °C during transport to the laboratory. The InPouch™ cultures were incubated at 37 °C and examined once a day for 5 days for the presence of trichomonads under light microscopy. Positive cultures were subcultured for cryopreservation and DNA extraction and molecular characterization as previously described (Girard et al., 2014a). If birds could not be sampled within 24 h after death, the carcass was stored in a freezer (−20 °C) until submitted for exam. Frozen carcasses were thawed at 4 °C for 24–48 h. Lesion tissues were sampled by excision, placed in a cryovial, and stored at −80 °C until molecular analysis. DNA was amplified and sequenced using primers targeting the ITS1/5.8S rRNA/ITS2 and Fe-hydrogenase loci and sequence analysis was performed by alignment to published trichomonad sequences in GenBank (Girard et al., 2014a). Amplicons whose sequences were difficult to interpret using Geneious Pro v. 5.34 sequence analysis software (Biomatters Ltd., Auckland, NZ) were cloned using the TOPO® TA cloning kit (Thermo Fisher Scientific, Waltham, MA), and 10 colonies per DNA isolate were chosen for further sequence analysis. All birds admitted to the study were assigned an 8-digit barcode beginning with CA0-.

Band-tailed pigeon mortality events were categorized by season based on the band-tailed pigeons annual cycle (Keppie and Braun, 2000). However, due to the annual variation in the timing of these activities as well as the temporal overlap of these activities by individual pigeons, the seasons were simplified into summer (May

through October) which primarily includes breeding and early fall migration and winter (November through April) which includes over-wintering and spring migration in California.

2.2. Spotted owl investigations

When an avian trichomonosis mortality event was detected in band-tailed pigeons, WIL notified wildlife rehabilitation centers and asked them to report infections in raptor species. Raptors admitted into rehabilitation centers with oral lesions typically died shortly after admission, or were euthanized due to poor prognosis. Carcasses were stored in a freezer until the post-mortem examination and lesion sample collection as previously described (Girard et al., 2014a). For one California spotted owl (CA015768), the carcass was submitted to WIL within 48 h after death and an oral swab was collected and inoculated into an InPouch™ for culturing of trichomonads. Immunohistochemistry (IHC) was performed on lesion tissue from this owl at CAHFS as previously described (Girard et al., 2014a). DNA was amplified and sequenced as described for band-tailed pigeons at the One Health Institute, University of California, Davis.

3. Results

3.1. Band-tailed pigeon mortality

Avian trichomonosis mortality events in band-tailed pigeons with spatial and temporal overlap with trichomonosis cases in spotted owls occurred during the winter of 2011–2012 (Fig. 1A), the summer of 2014 (Fig. 1B), and the winter of 2014–2015 (Fig. 1C).

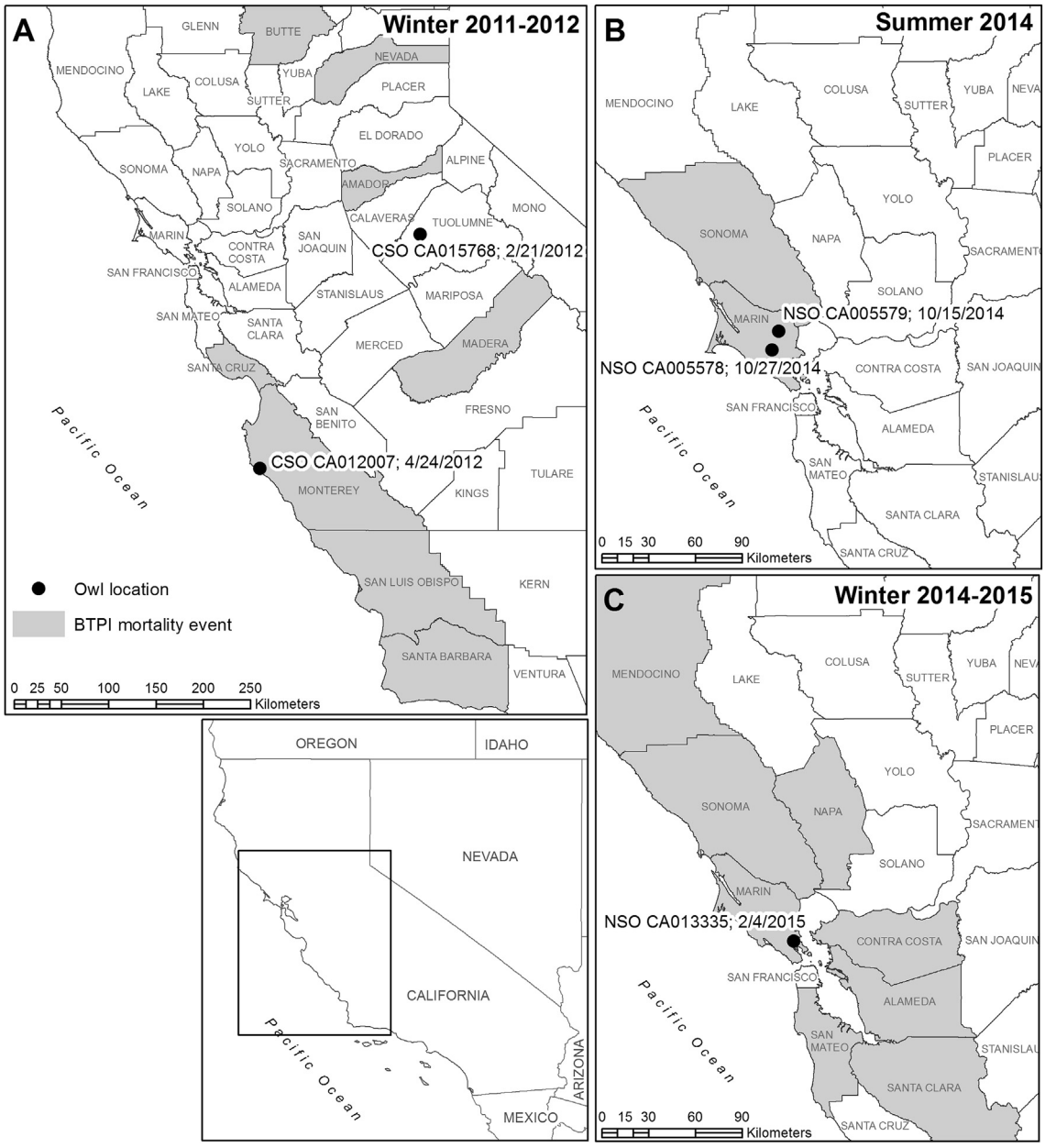

Fig. 1. Study area (black rectangle) in northern central California where spotted owls (*Strix occidentalis*) spatially and temporally overlapped with avian trichomonosis mortality events involving band-tailed pigeons (*Patagioenas fasciata monilis*). Counties shaded gray are those in which trichomonosis mortality events were detected in band-tailed pigeons (BTPI). Locations (black dots) of California spotted owls (CSO; *S. o. occidentalis*) and Northern spotted owls (NSO; *S. o. caurina*) diagnosed with avian trichomonosis during mortality events in band-tailed pigeons in winter 2011–2012 (A), summer 2014 (B), and winter 2014–2015 (C).

Fig. 1A–C shows the locations of spotted owls with avian trichomonosis in relation to counties with band-tailed pigeon mortality events reported here and previously investigated (Girard et al., 2014b; Rogers et al., 2016).

Trichomonad DNA was isolated from 25 band-tailed pigeons during the mortality event in Monterey County from January through March 2012 (Fig. 1A; Girard et al., 2014b). Fourteen pigeons were infected with *T. gallinae* subtype A2, 7 were infected with an un-typed *T. gallinae*, 3 were infected with *T. stableri*, and 1 was co-infected with *T. gallinae* A2 and *T. stableri* (Table 1). Band-tailed pigeon mortality also was detected in Madera, Amador, Nevada, and Butte counties between January and March 2012 (Fig. 1A; Girard et al., 2014b). Trichomonad DNA was isolated from 20 pigeons collected from Madera County (Girard et al., 2014b). Nineteen pigeons were infected with *T. gallinae* subtype A2 and 1 was co-infected with *T. gallinae* A2 and *T. stableri* (Table 1). Fewer band-tailed pigeons were collected for post-mortem examination from Amador, Nevada, and Butte counties and the parasites isolated from dead pigeons were exclusively *T. gallinae* subtype A2 (Girard et al., 2014b).

Mortality events were documented in Marin, Sonoma, and Los Angeles counties in summer, 2014 between May and October (Fig. 1B). During these events, 4 band-tailed pigeon carcasses were collected from Sonoma County, 16 from Marin County, and 6 from Los Angeles County. Eight of the Marin County pigeons were chosen for trichomonad isolation and genetic analysis. Of these, 6 were infected with *T. gallinae* subtype A2 and 2 with *T. gallinae* that could not be fine-typed (Table 1).

Mortality events were identified again during the winter in multiple counties from late November 2014 through April 2015 (Fig. 1C). Seven band-tailed pigeon carcasses collected during this period were selected for trichomonad isolation from Marin County and 1 from bordering Sonoma County. Six of the Marin County pigeons and the 1 Sonoma County pigeon were infected with *T. gallinae* subtype A2 and 1 Marin County pigeon was co-infected with an un-typed *T. gallinae* and *T. stableri* (Table 1).

3.2. California spotted owl infections

In total, five individual spotted owls were detected with avian trichomonosis between 2011 and 2015. Two were California spotted owls admitted into wildlife rehabilitation centers with suspected trichomonosis during winter 2011–2012 (Fig. 1A). Owl CA015768 was found in Tuolumne County and admitted to Rose Wolf Rehabilitation Center (Sonora, CA) on 21 February 2012 (Fig. 1A). As seen in Fig. 1A, Tuolumne County is located in the Sierra Nevada Mountain Range, to the north of Madera County and south of Amador County, where trichomonosis outbreaks in band-tailed pigeons were ongoing at the time of the owl carcass collection. The owl died on 23 February 2012 and was submitted to WIL within 48 h; an oral swab was collected and used to inoculate an InPouch™ device. Owl CA015768 was an adult female in thin body condition. Caseonecrotic lesions were noted across the hard palate from the caudal portion of the cloanal slit to the anterior pharynx. Lesions extended through the oral mucosa into the underlying muscle as well as into the choana and sinuses. The left and right inner ears had unilateral necrosis and florid inflammation with heterophils in the internal ear.

Owl CA012007 was found in Monterey County and admitted to the SPCA for Monterey County (Salinas, CA) on 24 April 2012 (Fig. 1A) and died overnight. This adult male owl was in thin body condition with caseonecrotic lesions extending across the upper palate. Avian trichomonosis was confirmed in both owls by PCR and also by IHC (in CA015768; Fig. 2). Trichomonads infecting both owls were identified as the same genotype most commonly found in band-tailed pigeons dying in the respective region: *T. gallinae* subtype A2 (Table 1).

3.3. Northern spotted owl infections

Two northern spotted owls were found in Marin County with suspected avian trichomonosis and were admitted to WildCare (San Rafael, CA) during summer 2014. Owl CA005579 was an adult in thin body condition found on 12 October 2014 (Fig. 1B). Caseonecrotic lesions of the oropharynx and tongue were noted during the intake exam by WildCare staff. The owl died on 15 October 2014 and the carcass was disposed of before a post-mortem examination could be performed and lesion samples collected for genetic analysis; the sex of the owl was undetermined. Owl CA005578 was found on 27 October 2014 (Fig. 1B) and was euthanized on 30

Table 1
Trichomonas spp. infecting band-tailed pigeons (*Patagioenas fasciata monilis*) and California spotted owls (*Strix occidentalis occidentalis*) recovered during avian trichomonosis epidemics involving band-tailed pigeons in California, during winter 2011–2012 and band-tailed pigeons and Northern spotted owls (*S. o. occidentalis*) during summer 2014 and winter 2014–2015.

County	Avian species	Sample collection (mm/yyyy)	Genotype				
			T. gallinae			Dual infection	
			FeH subtype A2	Un-typed	*T. stableri*	FeH subtype A2/ *T. stableri*	FeH subtype un-typed/ *T. stableri*
Monterey	Band-tailed Pigeon[a]	01/2012–03/2012	14	7	3	1	0
Monterey	California Spotted Owl (CA012007)	04/2012	1	0	0	0	0
Madera	Band-tailed Pigeon[a]	01/2012–03/2012	19	0	0	1	0
Tuolumne	California Spotted Owl (CA015768)	02/2012	1	0	0	0	0
Marin	Band-tailed Pigeon	05/2014–10/2014	6	2	0	0	0
Marin	Northern Spotted Owl						
	(CA005579)	10/2014	NT	NT	NT	NT	NT
	(CA005578)	10/2014	1	0	0	0	0
Sonoma	Band-tailed Pigeon	11/2014–04/2015	1	0	0	0	0
Marin	Band-tailed Pigeon	11/2014–04/2015	6	0	0	0	1
Marin	Northern Spotted Owl (CA013335)	02/2015	1	0	0	0	0

FeH, Fe-hydrogenase; NT, not typed.
[a] Girard et al., 2014b.

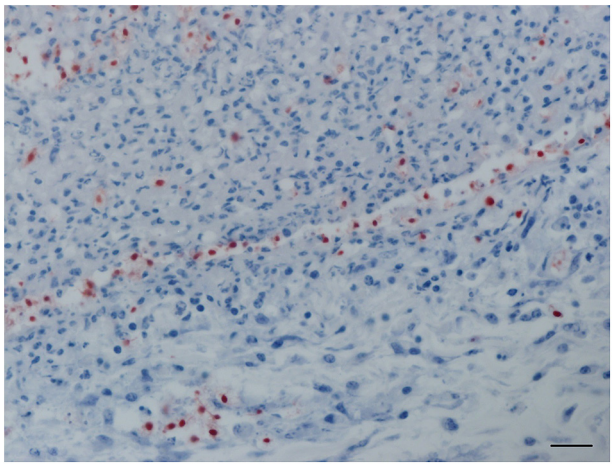

Fig. 2. Immunohistochemical staining of trichomonad antigen (red) in the oral tissue of California spotted owl (*Strix occidentalis occidentalis*) CA015768 recovered during an avian trichomonosis mortality event involving band-tailed pigeons (*Patagioenas fasciata monilis*) in Tuolumne County, California during winter 2011–2012. Scale bar is 10 μm.

October 2014. Owl CA005578 was an adult male in thin body condition with severe caseonecrotic lesions that extended across the upper palate and oral mucosa into the underlying muscle (Fig. 3). Additionally, CA005578 had lesions extending into the choana, sinuses, and inner ears as well as the bone at the base of the skull. Avian trichomonosis was confirmed by PCR in CA005578 and trichomonads were identified as *T. gallinae* subtype A2, the primary genotype causing mortality in band-tailed pigeons sampled in Marin County in summer 2014 (Table 1).

Lastly, one northern spotted owl was found in Marin County with suspected avian trichomonosis and was admitted to WildCare (San Rafael, CA) during winter 2014–2015. Owl CA013335 was found on 4 February 2015 (Fig. 1C) and was euthanized 5 days after admission due to the severity of infection. Owl CA013335 was an adult male in thin body condition with caseonecrotic lesions that extended across the upper palate and oral mucosa into the underlying muscle. Avian trichomonosis was confirmed by PCR in CA013335 and trichomonads were identified as *T. gallinae* subtype A2, the predominant genotype causing mortality in band-tailed pigeons sampled in Marin and Sonoma counties in winter 2014–2015 (Table 1).

Fig. 3. Caseonecrotic lesions in the oral cavity (white arrowhead) of Northern spotted owl (*Strix occidentalis caurina*) CA005578 recovered during an avian trichomonosis mortality event involving band-tailed pigeons (*Patagioenas fasciata monilis*) in Marin County, California during summer 2014.

4. Discussion

Investigation of five cases of avian trichomonosis in California spotted owls and northern spotted owls between 2011 and 2015 revealed that *T. gallinae* infections coincided in space and time with epidemic mortality events in band-tailed pigeons at three different time periods and geographic locations within northern central California. Furthermore, the genotype responsible for mortality in spotted owls was *T. gallinae* subtype A2, the same genotype responsible for concurrent epidemics in band-tailed pigeons. Taken together, these data provide evidence that trichomonosis in spotted owls was likely a result of disease spillover from a columbid species, and that we have detected multiple pathogen spillover events in these sensitive predatory birds in recent years.

Trichomonas gallinae subtype A2, the predominant trichomonad detected in this study, is frequently isolated in Pacific Coast band-tailed pigeons, and is the only *T. gallinae* genotype isolated during epidemics in this species (Girard et al., 2014b). Although *T. gallinae* subtype A1 is commonly isolated in passerines, columbids, and raptors in Europe (Chi et al., 2013; Lawson et al., 2011; Robinson et al., 2010), it only has been isolated from band-tailed pigeons during non-epidemic infections (Girard et al., 2014b). Although *T. stableri* was detected in a few band-tailed pigeons during two of the epidemics discussed in this study, it was not isolated in diseased owls, presumably because the prevalence was low in the potential prey species compared to *T. gallinae*. Further molecular and phenotypic characterization of the diverse avian trichomonads that have already been identified in California and beyond (Gerhold et al., 2008; Girard et al., 2014a, 2014b) is needed to understand the risk of transmission between raptors and their potential prey during epidemics.

Although spotted owls primarily prey on small mammals (Thrailkill and Bias, 1989; Ward et al., 1998), the documentation of avian trichomonosis in these owls suggests that they might opportunistically feed on birds and subsequently be exposed to diseases or contaminants found in avian prey. The remains of avian prey have been observed in spotted owl pellets, although the avian remains are rarely identified to species (Bond et al., 2013; Smith et al., 1999; Ward et al., 1998); however, Forsman et al. (2011) did identify band-tailed pigeon remains in pellets from northern spotted owls in Washington. During epidemics in band-tailed pigeons there is a relative abundance of moribund pigeons available to predators and/or recently dead pigeons available for scavengers, both of which may be a source of infection for these owls. While not documented in spotted owls, scavenging does occur in other owl species (Allen and Taylor, 2013; Kostecke et al., 2001). *Trichomonas* spp. parasites can remain viable in avian carcasses for at least 8 h after death and up to 24 h under appropriate environmental conditions allowing ample time for transmission (Erwin et al., 2000). Similar to columbid species, spotted owls with trichomonosis developed severe caseonecrotic lesions affecting the upper palate, and occasionally the ears and sinuses, leading to emaciation and death. The significance of avian trichomonosis in either northern or California spotted owls is currently unknown but has the potential to be a locally important cause of mortality, particularly during

epidemics in band-tailed pigeons.

Avian trichomonosis epidemics in band-tailed pigeons have been documented in California since the mid-1940s and have been reported with increasing frequency in recent years with nearly annual winter events contributing to long-term population decline (Rogers et al., 2016). In some cases, epidemics in band-tailed pigeons may go undetected when occurring in relatively remote areas or when smaller numbers of pigeons are involved. Similarly, an individual owl in this environment is even less likely to be encountered and reported, leading to under-detection of this disease threat. Northern and California spotted owls have been designated as sensitive species both federally and within California, with populations declining primarily in response to habitat loss and alteration (Gutierrez et al., 1995), although disease threats for these owls remain generally unknown. Significant impacts to raptor populations from avian trichomonosis have not yet been documented; however, certain raptor species that provision chicks with doves and pigeons have experienced high infection rates and mortality (Boal, 1997; Cooper and Petty, 1988; Real et al., 2000). Interestingly, raptors that opportunistically feed on birds may be at a higher risk of developing upper digestive tract lesions than raptors that regularly eat birds (Martinez-Herrero et al., 2014). Since band-tailed pigeon mortality events typically occur in the winter, the risk of infection is highest for adult spotted owls during this time, and potentially even the California threatened great gray owl (*S. nebulosa*) given their range overlap with band-tailed pigeons. However, during this study, avian trichomonosis mortality events in band-tailed pigeons were documented for the first time during the summer in 2014 indicating that adult owls as well as their chicks are at risk of *Trichomonas* spp. infection.

The unusual occurrence of a summer trichomonosis mortality event may have been related to the historic drought occurring in California at the time. By the end of July 2014, California was entering a third year of drought and over 58% of the state was classified as being in exceptional drought according to the National Drought Mitigation Center (http://droughtmonitor.unl.edu). Avian trichomonosis outbreaks in band-tailed pigeons tend to occur during winters with warmer and drier conditions (Rogers et al., 2016) possibly related to enhanced parasite viability or transmission opportunities. During summer, band-tailed pigeons are more dispersed for breeding and flock sizes are generally smaller than in the winter (Keppie and Braun, 2000). The extremely dry conditions during summer 2014 may have driven pigeons to congregate at available water sources, allowing for increased transmission among susceptible birds and subsequent detection by foraging owls. If weather extremes in California become more common as a result of climate change (Bell et al., 2004), avian trichomonosis has the potential to further impact both prey and predator species. Additionally, avian trichomonosis has recently emerged in several non-columbid species (Anderson et al., 2009; Robinson et al., 2010; Lehikoinen et al., 2013), suggesting increased risk of exposure in raptors that consume birds either regularly, or opportunistically, as infection spreads to more avian species (Chi et al., 2013).

Increased surveillance of *Trichomonas* spp. infection in spotted owls and other susceptible raptors, especially during epidemics in band-tailed pigeons and other prey, will improve our ability to evaluate the population health impact of avian trichomonosis in predatory species. Equally important is the continued efforts to genetically type trichomonad parasites isolated in raptors and their potential prey in order to elucidate transmission routes. Monitoring these owls only during the breeding season, as often occurs in demographic studies, would underestimate the importance of this disease during other times of the year. Ultimately, if infection risk is to be mitigated in these sensitive owl species, management considerations for band-tailed pigeons must find ways to minimize infection, especially during dry years, which will thereby reduce spillover into susceptible species.

Conflict of interest

The authors declare no conflict of interest.

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References

Allen, M.L., Taylor, A.P., 2013. First record of scavenging by a western screech owl (*Megascops kennicottii*). Wilson J. Ornithol. 125, 417–419.

Anderson, N.L., Grahn, R.A., Van Hoosear, K., Bondurant, R.H., 2009. Studies of trichomonad protozoa in free ranging songbirds: prevalence of *Trichomonas gallinae* in house finches (*Capodacus mexicanus*) and corvids and a novel trichomonad in mockingbirds (*Mimus polyglottos*). Vet. Parasitol. 161, 178–186.

Bell, J.L., Sloan, L.C., Snyder, M.A., 2004. Regional changes in extreme climatic events: a future climate scenario. J. Clim. 17, 81–87.

Boal, C.W., 1997. An Urban Environment as an Ecological Trap for Cooper's Hawks. Ph.D. Dissertation. University of Arizona, Tucson, Arizona, USA, 85 pp.

Bond, M.L., Lee, D.E., Siegel, R.B., Tingley, M.W., 2013. Diet and home-range size of California spotted owls in a burned forest. West. Birds 44, 114–126.

Chi, J.F., Lawson, B., Durrant, C., Beckmann, K., John, S., Alrefaei, A.F., Kirkbride, K., Bell, D.J., Cunningham, A.A., Tyler, K.M., 2013. The finch epidemic strain of *Trichomonas gallinae* is predominant in British non-passerines. Parasitology 1–12. http://dx.doi.org/10.1017/S0031182013000930.

Cole, R.A., 1999. Trichomoniasis. In: Friend, M., Franson, J.C. (Eds.), Field Manual of Wildlife Diseases: General Field Procedures and Diseases of Birds. U.S. Department of Interior, U.S. Geological Survey, Washington, D.C., USA, pp. 201–206.

Cooper, J.E., Petty, S.J., 1988. Trichomoniasis in free-living goshawks (*Accipiter gentilis gentilis*) from Great Britain. J. Wildl. Dis. 24, 80–87.

Davis, J.N., Gould Jr., G.I., 2008. *California spotted owl (*Strix occidentalis occidentalis*).* In: Shuford, W.D., Gardali, T. (Eds.), California Bird Species of Special Concern: a Ranked Assessment of Species, Subspecies, and Distinct Populations of Birds of Immediate Conservation Concern in California. Studies of Western Birds 1. Western Field Ornithologists, Camarillo, California, USA and California Department of Fish and Game, Sacramento, California, USA, pp. 227–233.

Erwin, K.G., Kloss, C., Lyles, J., Felderhoff, J., Fedynich, A.M., Henke, S.E., Roberson, J.A., 2000. Survival of *Trichomonas gallinae* in white-winged dove carcasses. J. Wildl. Dis. 36, 551–554.

Forrester, D.J., Foster, G.W., 2008. Trichomonosis. In: Atkinson, C.T., Thomas, J.J., Hunter, D.B. (Eds.), Parasitic Diseases of Wild Birds. Wiley-Blackwell, Ames, IA, USA, pp. 120–153.

Forsman, E.D., Otto, I.A., Sovern, S.G., Taylor, M., Hays, D.W., Allen, H., Roberts, S.L., Seaman, D.E., 2011. Spatial and temporal variation in diets of spotted owls in Washington. J. Raptor Res. 35, 141–150.

Forzan, M.J., Vanderstichel, R., Melekhovets, Y.F., McBurney, S., 2010. Trichomoniasis in finches from the Canadian Maritime provinces - an emerging disease. Can. Vet. J. 51, 391–396.

Gerhold, R.W., Yabsley, M.J., Smith, A.J., Ostergaard, E., Mannan, W., Cann, J.D., Fischer, J.R., 2008. Molecular characterization of the *Trichomonas gallinae* morphologic complex in the United States. J. Parasitol. 94, 1335–1341.

Girard, Y.G., Rogers, K.H., Gerhold, R., Land, K.M., Lenaghan, S.C., Woods, L.W.,

Haberkern, N., Hopper, M., Cann, J.D., Johnson, C.K., 2014a. *Trichomonas stableri* n. sp., an agent of trichomonosis in Pacific Coast band-tailed pigeons (*Patagioenas fasciata monilis*). Int. J. Parasitol. Parasites Wildl. 3, 32–40.

Girard, Y.G., Rogers, K.H., Woods, L.W., Chouicha, N., Miller, W.A., Johnson, C.K., 2014b. Dual-pathogen etiology of avian trichomonosis in a declining band-tailed pigeon population. Infect. Genet. Evol. 24, 146–156.

Grabensteiner, E., Bilic, I., Kolbe, T., Hess, M., 2010. Molecular analysis of clonal trichomonad isolates indicate the existence of heterogenic species present in different birds and within the same host. Vet. Parasitol. 172, 53–64.

Gutierrez, R.J., Franklin, A.B., Lahaye, W.S., 1995. Spotted owl (*Strix occidentalis*). In: Poole, A. (Ed.), The Birds of North America Online. Cornell Lab of Ornithology, Ithaca, NY, USA. Available online. http://bna.birds.cornell/bna/species/179.

Keppie, D.M., Braun, C.E., 2000. Band-tailed pigeon (*Patagioenas fasciata*). In: Poole, A. (Ed.), The Birds of North America Online. Cornell Lab of Ornithology, Ithaca, NY, USA. Available online. http://bna.birds.cornell.edu/bna/species/530.

Kostecke, R.M., Linz, G.M., Bleier, W.J., 2001. Survival of avian carcasses and photographic evidence of predators and scavengers. J. Field Ornithol. 72, 439–447.

Krone, O., Altenkamp, R., Kenntner, N., 2005. Prevalence of *Trichomonas gallinae* in northern goshawks from the Berlin area of northeastern Germany. J. Wildl. Dis. Issue 41, 304–309.

Lawson, B., Cunningham, A.A., Chantrey, J., Hughes, L.A., John, S.K., Bunbury, N., Bell, D.J., Tyler, K.M., 2011. A clonal strain of *Trichomonas gallinae* is the aetiologic agent of an emerging avian epidemic disease. Infect. Genet. Evol. 11, 1638–1645.

Lehikoinen, A., Lehikoinen, E., Valkama, J., Vaisanen, R.A., Isomursu, M., 2013. Impacts of trichomonosis epidemics on greenfinch *Ghloris chloris* and chaffinch *Fringilla coelebs* populations in Finland. Ibis 155, 357–366.

Martinez-Herrero, M.C., Sansano-Maestre, J., Lopez Marquez, I., Obon, E., Ponce, C., Gonzalez, J., Garijo-Toledo, M.M., Gomez-Munoz, M.T., 2014. Genetic characterization of oropharyngeal trichomonad isolates from wild birds indicates that genotype is associated with host species, diet and presence of pathognomonic lesions. Avian Pathol. 43, 535–546.

Real, J., Manosa, S., Munoz, E., 2000. Trichomoniasis in a Bonelli's eagle population in Spain. J. Wildl. Dis. 36, 64–70.

Robinson, R.A., Lawson, B., Toms, M.P., Peck, K.M., Kirkwood, J.K., Chantrey, J., Clatworthy, I.R., Evans, A.D., Hughes, L.A., Hutchinson, O.C., John, S.K., Pennycott, T.W., Perkins, M.W., Rowley, P.S., Simpson, V.R., Tyler, K.M., Cunningham, A.A., 2010. Emerging infectious disease leads to rapid population declines of common British birds. PLoS One 5, e12215.

Rogers, K.R., Girard, Y.A., Koenig, W.D., Johnson, C.K., 2016. Ecologic drivers and population impacts of avian trichomonosis mortality events in band-tailed pigeons (*Patagioenas fasciata*), California, USA. J. Wildl. Dis. 52, 484–494.

Sansano-Maestre, J., Garijo-Toledo, M.M., Gamez-Munoz, M.T., 2009. Prevalence and genotyping of *Trichomonas gallinae* in pigeons and birds of prey. Avian Pathol. 38, 201–207.

Smith, R.B., Peery, M.Z., Gutierrez, R.J., Lahaye, W.S., 1999. The relationship between spotted owl diet and reproductive success in the San Bernardino Mountains, California. Wilson Bull. 111, 22–29.

Stabler, R.M., Herman, C.M., 1951. Upper digestive tract trichomoniasis in mourning doves and other birds. Trans. North Am. Wildl. Conf. 16, 145–163.

Stromberg, M.R., Koenig, W.D., Walters, E.L., Schweisinger, J., 2008. Estimate of *Trichomonas gallinae*-induced mortality in band-tailed pigeons, Upper Carmel Valley, California. Winter 2006-2007 Wilson J. Ornithol. 120, 603–606.

Thrailkill, J., Bias, M.A., 1989. Diets of breeding and nonbreeding California spotted owls. J. Raptor Res. 23, 39–41.

Ward Jr., J.P., Gutierrez, R.J., Noon, B.R., 1998. Habitat selection by Northern spotted owls: the consequences of prey selection and distribution. Condor 100, 79–92.