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Ocular Plague (*Yersinia pestis*) in Mule Deer (*Odocoileus hemionus*) from Wyoming and Oregon

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ABSTRACT: Although plague is relatively rare in wild ungulates, this report describes ocular lesions associated with *Yersinia pestis* infection in three free-ranging mule deer (*Odocoileus hemionus*) from Wyoming and Oregon, USA. All deer were observed antemortem and seemed to be blind. Post-mortem examination revealed gross lesions of bilateral keratoconjunctivitis and/or panophthalmitis in the first two deer, but only partial retinal detachment in the third deer. Microscopically, all deer had moderate-to-severe necrotizing and fibrinopurulent endophthalmitis and varying degrees of keratoconjunctivitis with abundant intralesional coccobacilli. The lesions in the first (D1) and third deer (D3) suggested an acute course, whereas those in the second deer (D2) were subacute to chronic. *Yersinia pestis* was isolated from ocular tissue swabs or ocular fluids of D1 and D2, and it was demonstrated by immunohistochemistry within ocular lesions of D1 and D3. Although plague does not seem to be a major cause of morbidity or mortality in free-ranging mule deer, keratoconjunctivitis or pinkeye is relatively common in these animals and plague should be considered as a differential diagnosis in such cases, with appropriate precautions taken to protect the human and animal health.

Key words: Blindness, immunohistochemistry, keratoconjunctivitis, mule deer, *Odocoileus hemionus*, panophthalmitis, plague, *Yersinia pestis*.

Plague, caused by the bacterium *Yersinia pestis*, is a flea-borne disease of rodents, lagomorphs, some carnivores, and humans, and it has been described infrequently as a disease of ungulates (Poland and Barnes, 1979; Gasper and Watson, 2001). Sylvatic plague is maintained in reservoir rodent populations in semiarid areas of the western United States, whereas epizootics usually involve highly susceptible amplification rodent species (often prairie dogs, *Cynomys* spp.), and they sometimes spill over into a variety of susceptible nonrodent hosts, including mammalian carnivores and scavengers, raptors, humans (Hopkins and Gresbrink, 1982; Cully, 1991; Williams et al., 1994; Gese et al., 1997), and occasionally ungulates (Fedorov, 1960; Gordon et al., 1979; Thorne et al., 1987; Jessup et al., 1989).

Although plague is described as uncommon in domestic and wild ungulate species, several cases have been reported previously. Fedorov (1960) described varying susceptibility of camels (*Camelus bactrianus* and *Camelus dromedarius*) to *Y. pestis* in experimental inoculations and documented cases of camel carcasses infecting humans after slaughter and consumption in the former Union of Soviet Socialist Republics. Similar cases of plague, including more with presumptive transmission to humans, have been reported in other domestic livestock species, including camels and goats (*Capra* spp.) in Libya (Christie et al., 1980) and camels in Saudi Arabia (Bin Saeed et al., 2005). Plague also has been described in wild ungulates, including a free-ranging mule deer (*Odocoileus hemionus*) from Wyoming, USA. This deer demonstrated evidence of septicemia, with pneumonia and lymphadenitis, and the diagnosis was confirmed by culture and fluorescent antibody tests for *Y. pestis* (Thorne et al., 1987). There was no evidence of ocular involvement as described in the cases presented here.

The three deer presented were adults collected during 2004–2006 in Wyoming and Oregon, USA. All deer were observed alive by citizens or agency personnel before collection. Deer 1 (D1), from Wyoming, was a male observed by hunters in Albany County (41.1531°N, 105.2977°W).

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FIGURE 1. Mule deer (*Odocoileus hemionus*) from Wyoming with keratoconjunctivitis caused by *Yersinia pestis* infection.

The buck was alert and recumbent in a field, apparently blind, reluctant to stand, and ran into foreign objects when pressured. The deer was killed by gunshot to the thorax and submitted for necropsy to the Wyoming State Veterinary Laboratory (WSVL) (Laramie, Wyoming, USA).

At necropsy, D1 was an adult (estimated between 3–5 yr old, based on tooth eruption and wear) in excellent nutritional condition. Bilateral corneal opacity and mild reddening of the conjunctiva were observed, consistent with keratoconjunctivitis (Fig. 1). Significant trauma and hemorrhage associated with gunshot wounds effaced most normal tissue architecture in the pleural cavity, and no further significant gross lesions were observed.

Deer 2 (D2) was a male observed alive in Grant County, Oregon (44.4161°N, 118.9519°W), with bulging eyes and was killed by gunshot after observation that the buck seemed blind. A field necropsy was performed, and the head of the deer was submitted to the Veterinary Diagnostic Laboratory at Oregon State University (OSU VDL) (Corvallis, Oregon, USA). At necropsy, the deer was an adult in fair nutritional condition. Examination of the head revealed bilateral exophthalmos with associated keratoconjunctivitis and corneal ulceration (Fig. 2A). Bot fly larvae (*Cephenemyia* spp.) were present in the nasopharyngeal recess. No further significant gross lesions were observed in the head except for hemorrhage associated with the gunshot wound.

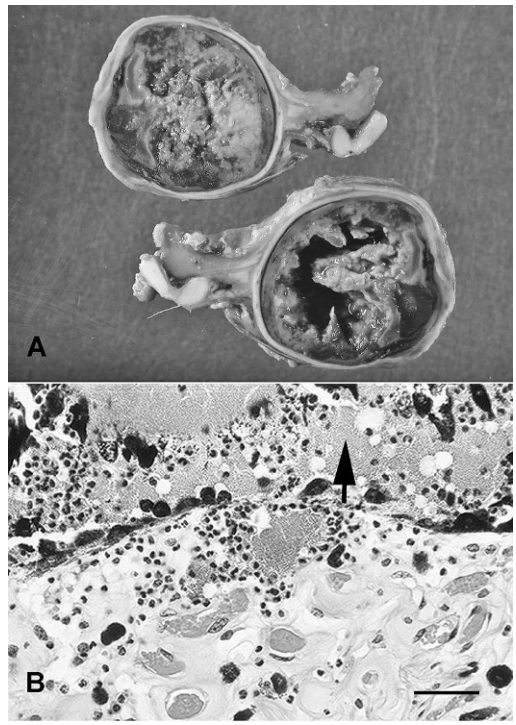


FIGURE 2. Eye from a mule deer (*Odocoileus hemionus*) with plague (*Yersinia pestis*). A. Sagittal section of the eye, demonstrating severe diffuse fibrinopurulent and necrotizing endophthalmitis. B. Photomicrograph of the choroid (top) and sclera (bottom) of the eye, with suppurative inflammation and numerous intralesional coccobacilli (arrow). H&E stain. Bar=75 μm.

Deer 3 (D3) also was killed via gunshot in Grant County, Oregon. This adult female was observed to be blind and ataxic but still associated with a group of 15 other mule deer. Field necropsy revealed the deer to be in fair body condition with mildly bulging eyes, but no internal lesions. The head was submitted to the OSU VDL for further testing. Approximately two dozen Pacific Coast ticks (*Dermacentor occidentalis*) were present and a few *Cephenemyia* spp. larvae again were observed. There were no corneal ulcers, and no exudate was observed within ocular chambers. A slight thickening and wrinkling of the anterior

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retina in one eye was interpreted as postmortem retinal detachment.

Microscopic examination of eyes from all deer revealed moderate-to-severe, multifocal-to-diffuse, bilateral, fibrinopurulent, and necrotizing panophthalmitis, with abundant intralesional coccobacilli (Fig. 2B). There was associated corneal necrosis, ulceration, neovascularization, and glaucomatous change of both eyes from D2, with diffuse retinal degeneration, indicating a more chronic clinical course than D1 and D3. Thrombosis and leukocytoclastic vasculitis of the uvea and retina were prominent features in D3, with exudate filling much of the space beneath the detached retina in one globe.

Additional microscopic lesions observed in D1 included moderate acute and multifocal necrotizing and fibrinopurulent pneumonia, multifocal mild necrotizing hepatitis, disseminated necrotizing lymphadenitis, mild tubulointerstitial and embolic nephritis, and disseminated microvascular thrombosis, all with similar abundant intralesional coccobacilli. In D2 and D3, only tissues from the head were available for microscopic examination. Multifocal mild nonsuppurative encephalitis was observed in D2, and mild lymphoplasmacytic infiltrates were seen in the choroid plexus of D3. These changes may have been incidental to plague.

Yersinia pestis was isolated from conjunctival sac and corneal swabs from D1, using Columbia agar with 5% sheep blood (Hardy Diagnostics, Santa Maria, California, USA) in 5% carbon dioxide (CO_2) with growth at 48 hr. *Yersinia pestis* was identified using the Biolog microbial identification and characterization system (Biolog, Inc., Hayward, California, USA) according to the manufacturer's recommendations. *Yersinia pestis* also was isolated and identified, using similar techniques, from ocular fluid aspirated from the posterior chamber of one eye in D2. Ocular tissues of D3 were not cultured but samples of meninges and retropharyngeal lymph node failed to yield growth of pathogenic bacteria.

Infection with *Y. pestis* was confirmed further using immunohistochemistry on sections of eyes from D1 and D3. Briefly, tissue sections were cut at 4–5 μm, mounted on glass slides (Superfrost® Plus, VWR International, Inc., West Chester, Pennsylvania, USA), deparaffinized and rehydrated, and treated with a commercial antigen retrieval solution (Target Retrieval Solution, Dako North America, Inc., Carpinteria, California, USA) for 35 min. The slides were stained using an automated immunohistochemistry machine (Dako North America, Inc.) with a commercial kit (LSAB+ System HRP Kit, Dako North America, Inc.), 3,3′-diaminobenzidine (DAB) chromogen (Dako North America, Inc.), and a rabbit polyclonal anti-*Y. pestis* antiserum (Lederle Laboratories, New York, New York, USA). This antiserum was made using live *Y. pestis* inoculated into rabbits, has strong reactivity against the *Y. pestis* F1 capsule antigen (Andrews, pers. comm.), and it does not react with a variety of other gram-negative bacteria in tissue sections in our laboratory (including *Escherichia coli*, *Salmonella typhimurium*, *Francisella tularensis*, and *Brucella abortus*; data not shown); the working dilution for the antibody was 1:2,000. Positive and negative controls, including deletion of primary antibody/use of secondary antibody alone and use of a nonsensical (anti-*F. tularensis* polyclonal) antibody were included in each immunohistochemistry run. This technique demonstrated intense labeling and staining of coccobacilli within the ocular lesions of both deer (Fig. 3) and staining of coccobacilli within lung, lymph node, and vascular lesions in D1.

In addition to plague, D1 was positive for chronic wasting disease (CWD) both by enzyme-linked immunosorbent assay (Hibler et al., 2003) and immunohistochemical (Miller and Williams, 2002) testing of retropharyngeal lymph node and obex region of the medulla oblongata. The deer was found within the CWD-endemic area of Wyoming and likely was in the preclinical stage of CWD due to light deposition of

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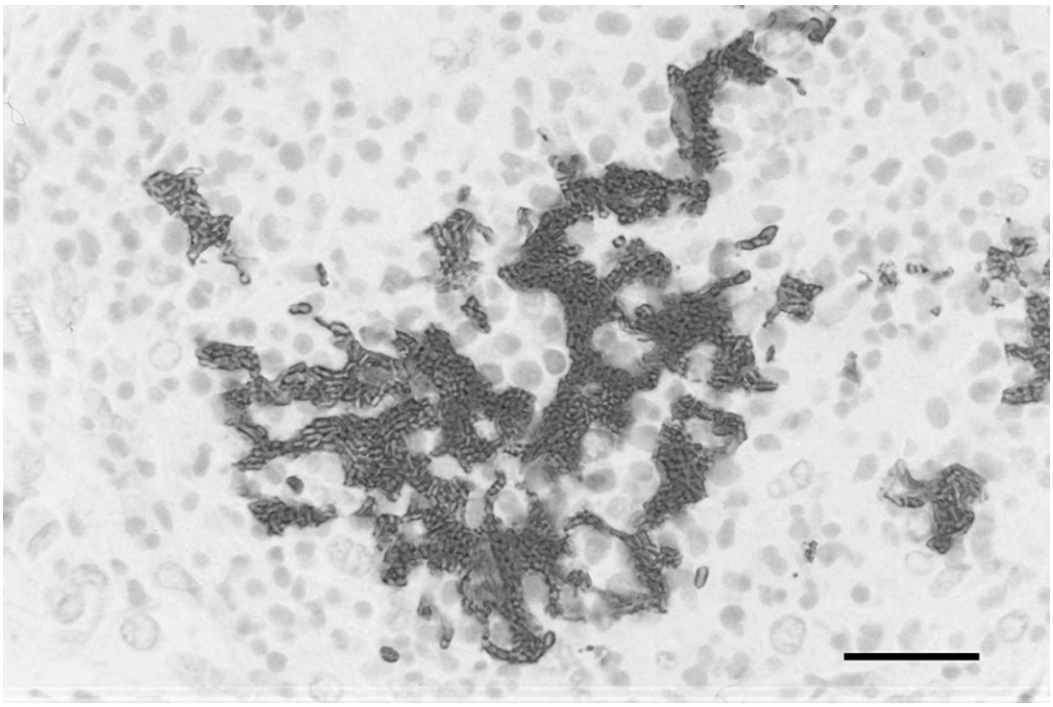


FIGURE 3. Immunohistochemical labeling and staining for *Yersinia pestis* in a section of eye from a mule deer (*Odocoileus hemionus*). All coccobacilli within the section label and stain intensely. Horseradish peroxidase method, DAB chromogen, and hematoxylin counterstain. Bar=30 μm.

pathogenic isoform of the prion protein, lack of spongiform change observed in the obex, and excellent nutritional condition.

Bilateral keratoconjunctivitis is relatively common in free-ranging mule deer, and although other viral, bacterial, and parasitic causes seem to be more common than plague (Taylor et al., 1996; Dubay et al., 2000), this is the second report of ocular plague in free-ranging deer in the United States. Jessup et al. (1989) reported bilateral ocular plague in a female black-tailed deer (*Odocoileus hemionus columbianus*) from southern Monterey County, California, USA, in August 1987. In most respects, that case resembled D2 described here, with more chronic changes, including exophthalmos (glaucomatous change), retinal degeneration, and extensive necrotizing and fibrinopurulent endophthalmitis. Several more cases of ocular plague also have been observed in mule deer in Colorado and Utah, USA, with keratoconjunctivitis, panophthalmitis, and sometimes with evidence (as with D1) of septicemia (Miller and Baldwin, pers. comm.). Included among these is one case confirmed at the WSVL by immunohistochemistry on ocular sections from a deer in Utah (data not shown).

Although there is no evidence that plague is a significant cause of morbidity or mortality in free-ranging mule deer, the cases reported do suggest that *Y. pestis* should be considered as a possible etiologic agent in deer with gross lesions consistent with keratoconjunctivitis and/or endophthalmitis/panophthalmitis. Although we do not know the route of transmission in deer with primary ocular plague, it is possible that these deer are infected across the cornea or conjunctiva by direct contact with infectious sources (e.g., rodent carcasses, mechanical vectors, or environmental fomites), but it also is possible that ocular infection and lesions more often are specific manifestations of systemic (septicemic) disease, acquired through the bite of

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biologic (flea[*Oropsylla taberculata cynomuris, Oropsylla idahoensis, Oropsylla labis,* and *Neopsylla inopina*]) vectors. Because plague is a serious zoonotic disease, education of wildlife professionals and deer hunters should be considered in plague enzootic areas, and appropriate precautions should be taken by wildlife disease professionals when examining deer with ocular lesions or a clinical history of blindness.

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