DOI: 10.7589/2015-01-014

Journal of Wildlife Diseases, 52(1), 2016, pp. 131–137

Brain, Craniofacial, and Dental Lesions of a Free-ranging Gray Wolf (*Canis lupus*) Implicated in a Human Attack in Minnesota, USA

Marc Schwabenlander,[1] Kevin Stepaniuk,[2] Michelle Carstensen,[3] and Aníbal G. Armién[1,4,5] [1]Veterinary Diagnostic Laboratory, College of Veterinary Medicine, University of Minnesota, 1333 Gortner Avenue, St. Paul, Minnesota 55108, USA; [2]Veterinary Clinical Sciences, College of Veterinary Medicine, University of Minnesota, 1365 Gortner Avenue, St. Paul, Minnesota 55108, USA; [3]Minnesota Department of Natural Resources, 5463C W Broadway, Forest Lake, Minnesota 55025, USA; [4]Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, 1365 Gortner Avenue, St. Paul, Minnesota 55108, USA; [5]Corresponding author (email: armie001@umn.edu)

ABSTRACT: We describe significant brain, craniofacial, and dental lesions in a free-ranging wolf (*Canis lupus*) involved in a human attack. On postmortem examination, the wolf presented asymmetric atrophy and bone remodeling affecting the mandible, incisive, maxilla, lacrimal, palatine, frontal, and ethmoid bones. There was an asymmetrical skeletal malocclusion and dental abnormalities including rotated, malpositioned, partially erupted teeth, and an odontogenic cyst associated with an unerupted canine tooth. Brain changes were bilateral loss and atrophy of extensive cortex regions including olfactory bulb, peduncles, and tract, and the frontal lobe. We highlight the relevance of a thorough postmortem examination of wildlife to elucidate disease-based abnormal behavior as the reason for human-animal conflict.

Key words: Abnormal behavior, brain injury, *Canis lupus*, craniofacial injury, malocclusion, odontogenic cyst, wolf attack.

On 24 August 2013, a report was provided to the Minnesota Department of Natural Resources that a 16-yr-old boy was injured in a free-ranging gray wolf (*Canis lupus*) bite incident early that morning in a campground on Lake Winnibigoshish in north central Minnesota, US. The boy sustained multiple puncture wounds and a laceration to his head, which were not life-threatening. Campground patrons indicated there recently were other incidents where an animal bit through tents, and a wolf was observed with coloration and markings matching the wolf described in the bite incident. On 26 August 2013 a male wolf, matching the description of the wolf in the attack, was caught in a foot-hold trap set by contracted trappers in the campground and then was euthanized. The wolf was submitted to the University of Minnesota Veterinary Diagnostic Laboratory (St. Paul, Minnesota, USA) for identity confirmation and postmortem examination.

The wolf was a 37-kg, approximately 1.5-yr-old intact male. On postmortem examination, the animal showed facial deformity. It was severely emaciated with a mild amount of subcutaneous and inner abdominal adipose tissue. The esophagus was empty, the stomach contained fish bones and scales, and the intestines had semifluid creamy digesta. The cause of death was gunshot.

The wolf's facial deformity corresponded to lesions affecting the craniofacial bones, with dental abnormalities grossly appreciable and supported by computed tomography (CT) imaging when compared with the control (Figs. 1A–F, 2A, B). There was asymmetrical atrophy and bone remodeling, with osteosclerosis affecting the mandible, incisive, maxilla, lacrimal, palatine, frontal, and ethmoid bones. There was a healed fissure with remodeling of the left frontal bone. Asymmetrical skeletal malocclusion and dental abnormalities included rotated, malpositioned, partially erupted teeth, and an odontogenic cyst associated with an unerupted canine tooth. The CT imaging applied to nonmacerated and macerated specimens demonstrated that ethmoid bone deformities resulted in an approximately 60% on the left and 20% on the right loss of rostral braincase space, representing similar percentage of brain tissue loss in the area of the frontal cerebrum and olfactory bulbs (Fig. 2A, inset). Frontal

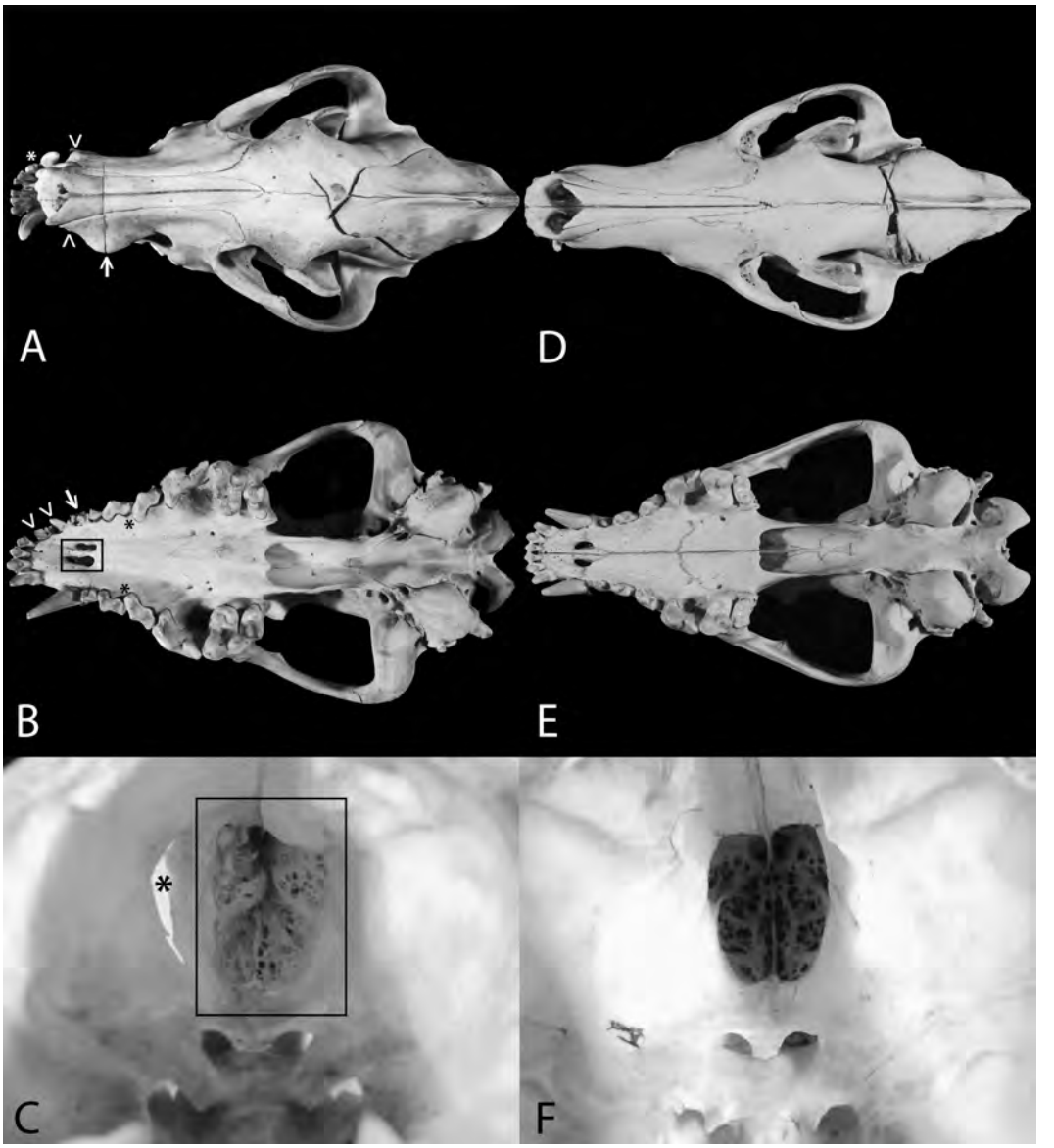

FIGURE 1. Macerated skull of a gray wolf (*Canis lupus*) with deformity compared to a control. (A) Dorsal view of affected wolf skull. Overall maxillary length was less than normal due to an underdeveloped incisive bone. Note marked alteration of geometry and density of the incisive, frontal, and maxillary bones. Note fusion of the left incisive and maxillary bones (arrowhead and odontogenic cyst [arrow]). The mandible under the maxilla shows asymmetry with the right side shorter than left (asterisk). (B) Ventral view of affected wolf skull with mandible removed. The rectangle outlines asymmetrically enlarged and deformed foramen incisive. Note unerupted canine tooth (arrow), partially erupted incisors (arrowheads), and rotation and abnormal contact of all interproximal surfaces of maxillary premolars (asterisks). Note fusion of incisive, maxillary, and palatine bones. (C) Frontal and ethmoid bones of affected wolf skull. Note asymmetric narrowing of the rostral cranial fossa at the level of the ethmoid bone, marked sclerosis and stenosis of the cribiform plate foramen (rectangle), and fissure of the left frontal bone (asterisk). (D) Dorsal view of control wolf skull. (E) Ventral view of control wolf skull with mandible removed. (F) Frontal and ethmoid bones within neurocranium of control wolf skull.

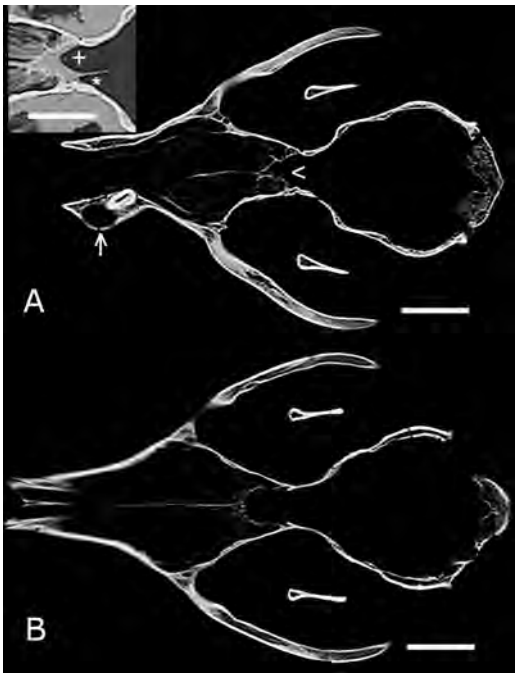

FIGURE 2. Computed tomography (CT) dorsal view of macerated gray wolf (*Canis lupus*) skull with deformities compared to a control. Bars=3 cm. (A) Note odontogenic cyst (arrow); deformity and sclerosis of cribiform plates (white arrowhead); and increased osseous density of maxillary, frontal, and zygomatic bones. Inset is CT dorsal view of fleshed (brain removed), affected gray wolf head. Reduced space of rostral neurocranium with variance from left (asterisk) to right (plus sign) side. (B) CT dorsal view of macerated, control gray wolf skull.

bones showed marked increased osseous density and frontal sinuses were deformed (Figs. 1C, 2A). There was increased osseous density of the left and right maxillary, frontal, and zygomatic bones (Fig. 2A).

The brain required great effort to remove properly from the braincase. The olfactory bulbs and frontal lobes seemed firmly attached to the meninges and the meninges firmly attached to surrounding bone. The brain presented with complete tissue loss of the left olfactory bulb and atrophy of left and right olfactory tracts, the right olfactory bulb, and both frontal cerebral lobes. No brain tissue remained in the braincase, only a discontinuous, thin, cystic wall-like membrane of the olfactory bulbs (tract) was observed. Destruction and atrophy of the frontal lobes were evident cranial to the sulcus suprasilvius affecting the gyri prorean and frontalis.

Brain samples were negative for rabies virus by immunofluorescent antibody testing at the Minnesota Department of Health, and Canine Neurological Panel PCR (*Anaplasma phagocytophilum*, *Borrelia burgdorferi*, Canine Distemper Virus, *Ehrlichia canis*, *Neospora hughesi* and *Neospora caninum*, *Rickettsia rickettssii*, *Toxoplasma gondii*, and West Nile virus) at the University of California–Davis Taqman Service, Davis, California, USA. Skeletal muscle from the wolf and samples from clothing used for first aid at the time of the bite incident had identical DNA profiles based on DNA analysis and match comparison at the University of California–Davis Veterinary Genetic Laboratory, confirming that the wolf in this report was involved in the bite incident and that it was a pure gray wolf and not a hybrid.

Organs and tissues were fixed in 10% neutral buffered formalin, processed and stained with H&E. A series of 2-mm coronal sections were examined histologically. A similar series of coronal sections from the brain of a previously healthy adult wolf were used as a control. The cruciate sulcus was used as the reference start point for the coronal sections of the control brain. An immunohistochemistry panel was carried out to assess detail of nerve tissue loss, repair, and inflammation response by using synaptophysin, glial fibrillary acidic protein (GFAP) and vimentin, and CD18, respectively. Immunohistochemical staining was performed on brain sections from both wolves using a peroxidase-based polymer system (EnVision™-HRP, Dako, Carpinteria, California, USA). Primary antibodies included synaptophysin (1:100, monoclonal; clone DAK-SYNAP, Dako), vimentin (1:500, monoclonal; clone V9, Dako), CD18 (1:20, monoclonal; clone CA16.3C10, Leukocyte Antigen Biology Laboratory, University of California–Davis), and GFAP (1:3,200, monoclonal; clone 1B4,

AbD Serotec, Raleigh, North Carolina, USA). Immunopositive reactions were visualized with the chromogen 3-amino-9-ethylcarbazole, and sections were counterstained with Mayer's hematoxylin (Leica Biosystem Richmond Inc., Richmond, Illinois, USA).

The lesions on the left olfactory bulb and frontal lobes were characterized by a loss of gray and white matter compared with the control (Fig. 3A–I). At the margin of the lost cortex, there was marked astroglial and microglia proliferation demonstrated by GFAP and CD18 immunohistochemistry (Fig. 3G–L). In the olfactory tract, in addition to a mild-to-moderate lymphocytic infiltration, there was vascular proliferation with marked fibroblastic response as demonstrated by vimentin immunostaining and polarized light (figure not shown). The right olfactory bulb, olfactory peduncle and tract, and frontal cerebral lobe had mild, diffuse white matter gliosis and loss of neuronal cell population as demonstrated by synaptophysin, GFAP, and CD18 (Fig. 3D–L). There was mild segmental perivascular lymphoplasmacytic infiltration and mild-to-moderate astrogliosis affecting the nuclei of the left and right amygdalae and parahypocampal cortex (figure not shown).

We describe significant brain, craniofacial, and dental lesions in the first (to our knowledge) confirmed wolf attack in Minnesota. In contrast to the majority of wolf-bite cases, there is strong evidence of biologic causation for this event.

We hypothesize that this wolf suffered craniofacial injury, possibly by an unspecified trauma, most likely a conspecific bite. This injury was healed at the time of the postmortem examination, indicating that it occurred at an early age. The craniofacial injury likely caused local, secondary inflammation that extended from the craniofacial soft and hard tissues to the frontal brain region. Although this hypothesis is difficult to confirm, evidence implicates trauma as the causal etiology. Traumatic injury in free-ranging, adult gray wolves is commonly seen in postmortem examination and is likely caused by large prey (e.g., moose, *Alces alces*; muskox, *Ovibos moschatus*), human interactions (e.g., vehicle collisions), or conspecific aggression (Wobeser 1992; Mörner et al. 2005). Of these common injuries, retrospective studies indicate that $<1\%$ of all bone injuries in adult wolves are skull associated and are most likely induced by prey interactions or conspecific aggression (Wobeser 1992; Mörner et al. 2005).

The wolf demonstrated a malocclusion characterized by a side-to-side maxillary-mandibular asymmetry that resulted in an abnormal dental masticatory apparatus. The initial injury likely caused direct and indirect lesions that altered the development of the mandible, incisive, maxilla, turbinate, lacrimal, palatine, frontal, and ethmoid bones and surrounding soft tissue. The overall asymmetrical skeletal malocclusion of the maxilla and mandibles in conjunction with dental abnormalities is most consistent with trauma. In cases of known trauma resulting in unerupted teeth and subsequent odontogenic (dentigerous) cyst development, dogs (*Canis lupus familiaris*) may also exhibit growth abnormalities that result in an asymmetric malocclusion. Furthermore, in dogs, a focal dentigerous cyst from an impacted or embedded tooth is generally not associated with an asymmetric skeletal malocclusion (D'Astous 2011). An associated extensive inflammatory process, possibly caused chronic damage and remodeling of the regional soft tissue and bone, altered the vasculature and resulted in ascending nerve tissue loss. Malocclusion, deviations in growth, and misconfiguration of the permanent teeth have been seen after the interruption of the innervation and the blood supply of the mandible in puppies who have suffered from rachitis, infectious diseases, or other illnesses during odontogenesis (Skopakowa and Skopakoff 1982).

Compromise in the dentition or bite of a wolf can impede food procurement. During times of decreased primary prey populations or when unable to capture typical prey, wolves may become omnivorous,

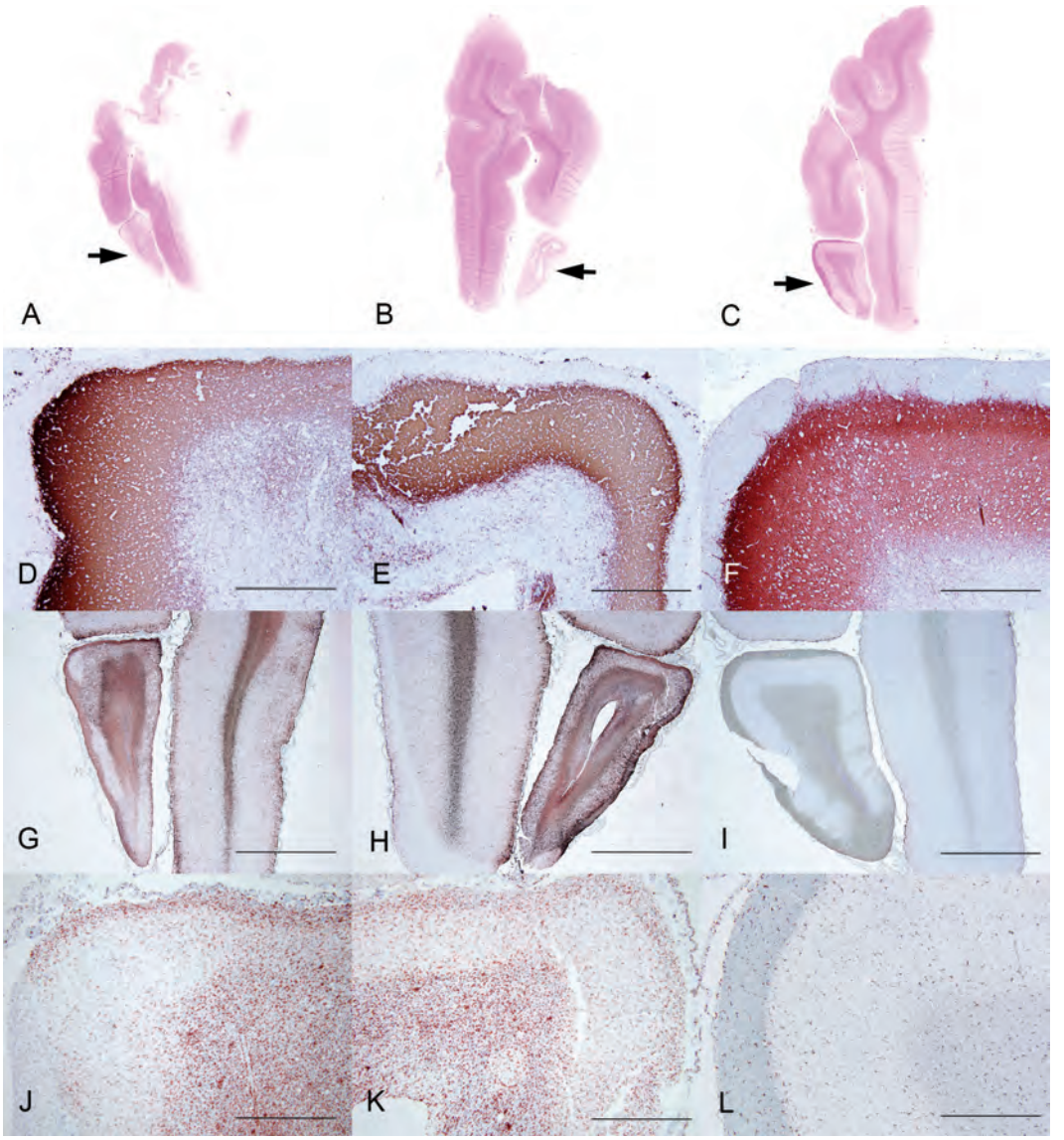

FIGURE 3. Brain of a gray wolf (*Canis lupus*) with marked lesion compared to a control. All bars=500 µm. (A) Affected wolf: coronal section of the left frontal lobe and olfactory peduncle, 10 mm cranial to the cruciate sulcus. Note marked loss and atrophy of brain tissue. Arrow indicates atrophic olfactory peduncle. H&E. (B) Affected wolf: coronal section of the right frontal lobe and olfactory peduncle, 10 mm cranial to the cruciate sulcus. Note moderate-to-marked atrophy of brain tissue. Arrow indicates atrophic olfactory peduncle. H&E. (C) Control wolf: coronal section of left frontal lobe and olfactory peduncle (arrow), 10 mm cranial to the cruciate sulcus. H&E. (D) Affected wolf: left olfactory peduncle. Atrophy of the cortex demonstrated by synaptophysin immunostain. Note irregular reduction of the thickness of the stained cortex due to loss of a large number of neurons and axonal and dendrite processes. (E) Affected wolf: right olfactory peduncle. Same features in (D). (F) Control wolf: left olfactory peduncle. Normal cortex thickness of the cortex demonstrated by synaptophysin immunostain. (G) Affected wolf: left olfactory peduncle and frontal cortex. Atrophy with marked astroglial proliferation and reaction. The overexpression of glial fibrillary acidic protein (GFAP) by astrocytes is a response to a chronic injury. GFAP immunostain. (H) Affected wolf: right olfactory peduncle and frontal cortex. Same features as in (H). GFAP immunostain. (I) Control wolf: left olfactory peduncle and frontal cortex. The cortex has small number and low expression of GFAP-positive cells consistent with quiescent astrocytes in a normal brain. GFAP immunostain. (J) Affected wolf: left olfactory peduncle. Atrophy with marked proliferation and reaction of CD18-expressing cells, presumably active microglia suggesting chronic inflammation. (K) Affected wolf: right olfactory peduncle. Same features as in (K). (L) Left olfactory peduncle: control wolf. The cortex has small numbers of CD18-expressing cells, presumably quiescent resident microglia in a normal brain.

taking advantage of the incisors' independent arrangement from the canines for ingestion of small, nonstruggling food items such as fruits, nuts, or small prey (Peterson and Ciucci 2003). The dental abnormalities in this wolf indicated a compromised ability to acquire food, in multiple facets, and contributed to emaciation from malnourishment. The facial changes may have also altered the wolf's communication abilities, communications that govern pack social behavior and survival. For example, eight facial expressions enable wolves to make fine discriminations of the mood and intent of conspecifics within a pack (Jacobs et al. 2003). Thus, this wolf's impairments, leading to adaptive survival strategies, may be one of the factors that triggered this attack, but not the primary cause. The wolf's dental abnormalities and compromised function may also be a primary reason the human victim escaped with minor injury.

Tissue loss, atrophy, and inflammation of the brain cortices seem to be a consequence of the craniofacial injury. Tissue loss and atrophy of the brain cortex was assessed by a combination of the gross, CT imaging, histopathologic, and immunohistochemical semiquantitative analysis. In addition, lymphoplasmaytic infiltration and overexpression of GFAP by astrocytes and expression of CD18 by proliferative and reactive microglia in affected areas indicate chronic brain tissue inflammatory and reparative response; they also indicate that the brain morphologic and physiologic damage is extended further than the quantifiable tissue loss. The lesions in the wolf's brain affecting olfactory bulbs, peduncles, and tracts and pyriform and frontal cortices suggest severe damage to the areas and circuitries associated with cognitive function, emotion, and olfaction. This conclusion is supported by naturally occurring and experimentally induced lesions in the frontal cortex and secondary circuitry remodeling to the limbic system in domestic canines that have demonstrated changes in behavior, probability-based prediction, inhibition, aggression, maintenance, and manipulation of complex learning, and spatial and conceptual memory (Jacobs et al. 2003; Christie et al. 2008). It has been theorized that wolves use spatial memory and learning to help locate geographically disparate prey (Hiestand 2011). The loss of this faculty would decrease a wolf's ability to seek prey. Similar functional changes are demonstrated in other species, including humans who suffer from genetic disorders and acute and chronic traumatic brain injuries in the frontotemporal region (Anderson et al. 2006). Minimal injury is needed to produce these changes, although reported damage ranges from focal, microscopic alteration to complete removal (Jacobs et al. 2003; Anderson et al. 2006; Christie et al. 2008; Morales-Medina et al. 2013). Furthermore, olfaction is crucial and provides data about prey, danger, and other wolves' identity; sex; breeding condition; social status; emotional state; age; condition; and diet (Harrington and Asa 2003). Wolves seem to use olfactory information to choose future actions (Harrington and Asa 2003). Experimentally olfactory bulbectomized rats have exhibited memory behavioral deficits, psychomotor retardation, learning deficits, decreased grooming behavior, and reduced social interaction, in part, due to nervous tissue and circuitry remodeling from the lack of olfactory bulb input (Morales-Medina et al. 2013). Even with potential partial olfaction remaining, the consequence of what was lost to this wolf was likely detrimental.

Wolf attacks on humans are extremely rare and well-documented reports of aggressive wolf encounters show that few have led to serious injury and tend to involve extenuating circumstances (McNay 2002). Mental, social, and food procurement disabilities of this wolf likely set the conditions for its reaction under unusual circumstances. Damage to the rhinal and frontal lobes likely prevented the wolf from evaluating the consequences of an attack on a nonprey species. Our findings emphasize the importance of a complete postmortem examination to identify the

underlying causes of negative human-animal interactions.

We thank Erika Butler for involvement and Ronda Aho and Jan Shivers for the histologic and immunohistochemistry preparations.

LITERATURE CITED

Anderson SW, Barrash J, Bechara A, Tranel D. 2006. Impairments of emotion and real-world complex behavior following childhood- or adult-onset damage to ventromedial prefrontal cortex. *J Int Neuropsychol Soc* 12:224–235.

Christie LA, Saunders RC, Kowalska DM, MacKay WA, Head E, Cotman CW. 2008. Rhinal and dorsolateral prefrontal cortex lesions produce selective impairments in object and spatial learning and memory in canines. *J Comp Neurol* 511:257–270.

D'Astous J. 2011. An overview of dentigerous cysts in dogs and cats. *Can Vet J* 52:905–907.

Harrington FH, Asa CS. 2003. Wolf communication. In: *Wolves: Behavior, ecology, and conservation*, Mech LD, Boitoni L, editors. University of Chicago Press, Chicago, Illinois, pp. 80–88.

Hiestand LA. 2011. Comparison of problem-solving and apatial orientation in the wolf (*Canis lupus*) and dog (*Canis familiaris*). *Behav Gene* 41:840–857.

Jacobs C, De Keuster T, Simoens P. 2003. Assessing the pathological extent of aggressive behaviour in dogs. A review of the literature. *Vet Q* 25:53–60.

McNay ME. 2002. A case history of wolf-human encounters in Alaska and Canada. *Wildlife Technical Bulletin 13*. Alaska Department of Fish and Game, Juneau, Alaska. http://fwp.mt.gov/fwpDoc.html?id=10996. Accessed September 2014.

Morales-Medina JC, Juarez I, Venancio-García E, Cabrera SN, Menard C, Yu W, Flores G, Mechawar N, Quirion R. 2013. Impaired structural hippocampal plasticity is associated with emotional and memory deficits in the olfactory bulbectomized rat. *Neuroscience* 236:233–243.

Mörner T, Eriksson H, Bröjer C. 2005. Diseases and mortality in free-ranging brown bear (*Ursus arctos*), gray wolf (*Canis lupus*), and wolverine (*Gulo gulo*) in Sweden. *J Wildl Dis* 41:298–303.

Peterson RO, Ciucci P. 2003. The wolf as a carnivore. In: *Wolves: Behavior, ecology, and conservation*, Mech LD, Boitoni, L, editors. University Of Chicago Press, Chicago, Illinois, pp. 104–130.

Skopakowa K, Skopakoff C. 1982. Deviations of bite, growth and arrangement of permanent teeth in dogs following interruption of innervation and blood supply to the lower jaw in dogs. *Anat Anz* 152:39–45.

Wobeser G. 1992. Traumatic, degenerative, and developmental lesions in wolves and coyotes from Saskatchewan. *J Wildl Dis* 28:268–275.

Submitted for publication 19 January 2015.
Accepted 26 June 2015.