

# Reovirus associated with mortality of an Upland Sandpiper

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Population dynamics of waders are shaped by a range of different environmental factors including annual variation in food availability, climatic conditions, predation pressure, and the interaction of these factors with population density (Evans & Pienkowski 1984, Yalden & Pearce-Higgins 1997, Whitfield 2003). One factor that is poorly understood in most wildlife populations is the ecological role of pathogens and parasites (Friend *et al.* 2001). Disease dynamics are difficult to study in free-living birds because carcasses of small animals are usually consumed by scavengers and decompose rapidly (Osborne *et al.* 2000), and because large die-offs are rare events (Adams *et al.* 2003, Woodard *et al.* 1977). Nevertheless, diseases of waders may be of ecological and epidemiological importance, particularly if long-distance migrants are a vector for dispersal of zoonotic pathogens, such as West Nile virus (Komar 2003), avian influenza virus (Hurt *et al.* 2006, Makarova *et al.* 1999), avian cholera (Adams *et al.* 2003), and avian malaria (Mendes *et al.* 2005).

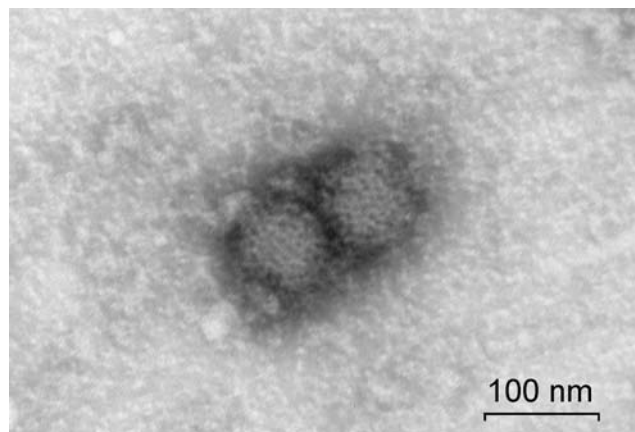
Here, we report isolation of a reovirus from the brain of a wild Upland Sandpiper *Bartramia longicauda* found dead in the field. Reoviruses are viruses of respiratory or enteric origin (REO), and are characterized by spherical virus particles 75 to 80 nm in size, a double-stranded RNA genome, and a double capsid protein shell (Read-Connole 2000, Ritchie & Carter 1995). Reoviruses are best known as a pathogen of domesticated or captive poultry, waterfowl and psittacines (Ritchie & Carter 1995, Robertson & Wilcox 1986), but have also been detected in wild populations of waders, waterfowl and other birds (Docherty *et al.* 1994, Hlinak *et al.* 2006, Hollmen *et al.* 2002).

We conducted a population study of Upland Sandpipers during April to August of 2002–2007 at Konza Prairie Biological Station in northeast Kansas, USA (39°05'N, 96°34'W). In this six-year period, we radio-marked 261 adult sandpipers by attaching transmitters with either a leg-loop harness ( $n = 203$ ) or adhesives ( $n = 58$ ). Mortality of sandpipers during the breeding season was infrequent, and the prob-

ability of surviving the ten-week breeding period was 0.875 in females and 0.965 in males (Mong & Sandercock 2007). We recovered eight carcasses of radio-marked birds during our six-year study, and evidence at recovery sites indicated that seven of eight dead sandpipers were killed by predators or scavenged after dying of unknown causes.

This case history is based on a single Upland Sandpiper (U.S. Fish & Wildlife Service band 1573-29956) that was captured and radio-marked on 19 May 2005 by BKS and AEC, and monitored for 30 days. We discovered the bird incubating a four-egg clutch on 25 May, and the clutch successfully hatched on 12 June. We relocated and observed the bird on three brood checks between 13–16 June. The bird exhibited normal behavior when approached by an observer, including flying, perching on fence posts, and giving vocalizations associated with brood attendance and defence of young. On 18 June, we recovered the intact dead bird lying on its side with the legs flexed and the head arched back over the spine. From inspection of the carcass at the recovery site, BKS and AEC discounted predation, collision and harness entanglement as possible causes of death. The six-day old young were not relocated and their fate was unknown.

The carcass was frozen and shipped on dry ice to the National Wildlife Health Center (U.S. Geological Survey) at Madison, Wisconsin for diagnostic examination. Upon receipt, the sandpiper was dissected by DEG and a range of cultures and tissue analyses were completed. External examination revealed no significant abnormalities. Internally, the bird was in fair body condition and in good post-mortem condition without decomposition. Internal gross examination revealed a small, atrophied spleen ( $1.5 \times 5.0$  mm), and reddish-black pasty material in the small intestine, presumed to be blood from intraluminal hemorrhaging. The bird had been initially sexed by molecular markers based on the *CHD*-gene (Griffiths *et al.* 1998, S.M. Wisely, Kansas State University), and its sex was confirmed to be male by inspection of the gonads during necropsy.



**Fig. 1.** Electron micrograph (negative contrast) of reovirus particles from isolates obtained from brain tissues of an Upland Sandpiper found dead under field conditions. The virions are approximately 70 nm in diameter and exhibit the typical double shelled morphology that is diagnostic of reoviruses.

Virus cultures of the brain prepared by HIP were positive for reovirus. Electron microscopy revealed virus particles with virions that were approximately 70 nm in diameter, and exhibited the typical double shelled morphology that is diagnostic of reoviruses (Fig. 1). No viruses were isolated from the lung, liver, kidney or small intestine. Other tissues from the dead sandpiper were negative for other pathogens and contaminants, including West Nile virus (brain, spleen, kidney), *Salmonella* (intestine), botulism type C (heart, liver), other bacteria (liver, intestine), and organophosphate or carbamate pesticides (brain).

Microscopical examinations of tissues by DEG revealed necrosis of lymphocytes in the gut-associated lymphoid tissues of Peyer's patches, Meckel's diverticulum, and caecal tonsils. Lymphocytic necrosis is a non-specific lesion with multiple causes that include reovirus infections, but may also be due to stress, intoxication, contaminants, or other types of viral infection (Green & Albers 1997). Nevertheless, we conclude that the Upland Sandpiper died with a reovirus in its brain, and the associated symptoms are consistent with reovirus infection as a cause of death.

To our knowledge, this article is the third report of isolation of a reovirus from a wild population of waders. A novel reovirus was implicated in large die-offs of American Woodcock (*Scolopax minor*) during the winters of 1989–90 and 1993–94 in the eastern United States (Docherty 1999, Docherty *et al.* 1994). Reo-like viruses have also been recovered from waders that were live-captured and sampled as passage migrants at Lake Guelpe, Germany (4 of 465 individuals,  $n = 19$  species, Hlinak *et al.* 2006). Of the four positive isolates, three originated from Dunlin *Calidris alpina* and one was from a Spotted Redshank *Tringa erythropus*.

Our findings extend previous reports of reoviruses in waders in three ways. First, our case is unusual because the reovirus was isolated from the brain tissues of the dead male Upland Sandpiper. Avian reoviruses are most frequently recovered from the digestive tract (waders and other birds: Hlinak *et al.* 2006, Ritchie & Carter 1995) or respiratory organs and skeletal joints (poultry: Robertson & Wilcox 1986). Interestingly, reoviruses were also isolated from the brains of sick and dying woodcocks, and were considered the cause of death in that species (Docherty *et al.* 1994). Recovery of reoviruses from the digestive tract suggests a fecal-oral route of transmission, but infection of nervous tissues may indicate a different, as yet unknown pathway. Second, the dead Upland Sandpiper was recovered during the breeding season. Previous isolates of reoviruses from waders were collected mainly from wintering or migrating birds (Hlinak *et al.* 2006). Last, the Upland Sandpiper exhibited apparently normal behavior, and was in fair body condition at the time of death. In the case of die-offs among woodcocks, dead birds infected with reoviruses were emaciated at death (Docherty *et al.* 1994).

The ecological significance of reoviruses for Upland Sandpipers remains unclear because mortality events are infrequent and difficult to detect under field conditions. In most species of waders, relatively few carcasses of dead birds have been tested for pathogens. We encourage wildlife biologists to submit freshly dead bird carcasses for diagnostic examination whenever feasible. Obtaining viral isolates from a broader range of organs, species, and habitats will contribute to a better understanding of the role of reoviruses as a pathogen in wild populations of waders and other birds.

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