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CCWHC Involvement with Botulism

Botulism has been a major problem in western North America during the 1990's, as reported in previous newsletters. In1997, an estimated 1.2 million birds died on three lakes used by molting birds in the prairie provinces, and about 0.5 million birds died on the Bear River Marshes in Utah. The CCWHC has always been involved in the diagnosis of botulism, but our role has been expanded dramatically in the past 18 months.

In January 1997, the Institute for Wetland and Waterfowl Research (IWWR, the research arm of Ducks Unlimited) and CCWHC Western/Northern region organized an interagency workshop on botulism in Saskatoon that drew managers and researchers together. One outcome was an agreement that the disease must be addressed on a regional rather than a local basis, and that there were great benefits in a cooperative approach. Another result was that during the summer of 1997, Trent Bollinger from Western/Northern region was involved with scientists from the Canadian Wildlife Service in a cooperative investigation at Old Wives Lake, SK, that developed methods for measuring mortality, and that documented the extent of mortality on that lake.

Because of the magnitude of losses to botulism, the Prairie Habitat Joint Venture (PHJV), an interagency group responsible for implementing the North American Waterfowl Management Plan in prairie Canada, asked CCWHC to organize a Working Group on Avian Botulism. The mandate of the group is to review current knowledge regarding botulism, to develop standardized techniques for surveillance and data collection, to identify information gaps and research needs, to evaluate current management techniques and develop methods to test their effectiveness, and to provide management recommendations to PHJV. The 12 member working group includes biologists experienced in dealing with outbreaks from provincial agencies and Ducks Unlimited, and scientists from IWWR, the Canadian Wildlife Service, the Canada Centre for Inland Waters, the U.S. National Wildlife Health Center, the U.S. Fish & Wildlife Service, Utah State University and two from CCWHC (Trent Bollinger and Gary Wobeser, who chairs the group).

The group met in January 1998, and identified three critical information gaps: 1) the impact of botulism on bird populations, 2) the effectiveness of carcass pickup as a management technique and, 3) the need to develop methods for assessing the risk of botulism on wetlands. The group made interim recommendations to PHJV for the summer of 1998 that included: overall coordination of investigative efforts at Pakowki, Whitewater and Old Wives Lakes; aerial surveys together with ground surveys to define the bird population at risk on these lakes, establishment of a regional investigative team to collect standardized information on mortality and effectiveness of carcass collection on the lakes, and collection of information from Canadian wetlands for validation of a risk assessment model developed by the US National Wildlife Health Center.

As a result of the recommendations, CCWHC was asked to prepare a detailed proposal for regional coordination, a regional investigative unit, and coordination of population surveys. That proposal has been funded by the partners within PHJV, with additional support being provided by the California Waterfowl Association. The support includes funds to hire a replacement

pathologist for the time Trent Bollinger will devote to the project as Regional Coordinator, funds to hire and equip a field investigative unit (Fig. 1) that will collect information at each lake at 2 week intervals throughout the summer, increased diagnostic effort, and improved communication among the agencies involved in botulism. The aerial surveys will be done by experienced US Fish & Wildlife Service crews at regular intervals. These activities will complement studies by other agencies at the lakes that include measurement of physical and chemical factors, occurrence and toxicity of blue-green algae, as well as using radiotelemetry and vaccination in conjunction with banding to measure mortality caused by botulism. Standardized forms developed by the Working Group will be used for recording information on all aspects of botulism throughout the prairies.

The Working Group is developing a model of the ecology of botulism that incorporates current knowledge; recommendations for assessing the population effects of botulism at the local, regional and continental level; improved methods for assessing whether carcass collection is effective in reducing mortality; and methods for improving communication regarding botulism; as well as identifying research priorities. The overall goal is to develop a system for adaptive management of this important disease. A final report to PHJV will be submitted during June, 1998. Based on the positive results gained by assembling expertise in the group, a recommendation will be that a working group on botulism should be continued and expanded to include at least the USA and Mexico, because botulism is a problem throughout the continent, as well as elsewhere in the world. CCWHC will continue to be actively involved in this evolving process.

Feature Article

The Value of a Complete Necropsy in Controversial Situations

One of the fundamental services that the CCWHC offers to its supporting agencies is ocesses. Often assistance is sought from ecologists to combine information on the biology of the animal with the laboratory finding in arriving at a final diagnosis.

The importance of a complete necropsy by an impartial, trained pathologist is most obvious in forensic cases, in which the results are to be used as evidence in legal proceedings. In these situations, the pathologist acts as an independent expert witness and not as an advocate for either side in the controversy. The same principle of impartial objectivity applies in many other situations, in which legal action is unlikely, but factual information is required for the "*court of public opinion"*. This is particularly true in those situations in which there is great public interest in why animals have died. In some cases, a judgement may have been made as to who or what is responsible for the problem, before any animals have been examined. The necropsy helps to ensure that blame is not attributed inappropriately.

In this and almost every past newsletter, we have described instances in which animals, were found through careful diagnostic examination to have succumbed to contaminants and toxins (barbiturates, lead, mercury, various pesticides, teflon, avitrol, salt, strychnine, antifreeze) or to other human activities, such as propeller wounds on whales. It is important in such instances, that substantive evidence is collected and catalogued, so that it can be used to reduce the probability

of further harm occurring in the future. In some of these cases, the necropsy confirmed a suspicion about the cause of death, while in others the animals were initially thought to have died of "natural causes".

Equally important are those situations in which the actual cause of mortality, as ascertained at necropsy, is different than the preconceived judgement. Some cases examined by CCWHC over the past few years illustrate this principle.

In July 1993, Ring-billed gulls (*Larus delawarensis*) found dead near two chemical factories in Quebec were presumed to have died of intoxication resulting from effluent from the industries, and this had been reported on the local television station. Specimens examined at the Quebec Regional Centre indicated that the birds died as a result of infection with *Salmonella typhimurium* bacteria and no evidence was found of poisoning (described in Vol. 3[1]).

In June 1996, Common Terns (*Sternus hirundo*) found dead in Kouchibouguac National Park were thought to have been killed by shooting but the lesions found at necropsy were not compatible with those seen in gunshot wounds. Predation by Great Horned Owls (*Bubo virgianus*) was thought to be the probable cause of death (described in Vol. 4[2]).

In September 1997, dead ciscoes (*Coregonus artedii*) were found floating in the water of a lake near a mine in northern Saskatchewan. It was suspected that toxic effluent from the mine was responsible for their death; however, at necropsy the fish were found to have severe infection within the heart by larval parasites (described in Vol. 5[1]).

In this issue, the necropsy of a large number of seal pups found dead on the coast of Prince Edward Island is reported. The presumption by some was that the animals had been killed by sealers on the ice; however, the 86 seals necropsied did not have injuries compatible with trauma caused by seal hunters. It appears that the animals died of natural causes and that some were subsequently fed upon by scavengers.

Other examples investigated recently by CCWHC in which the animals were thought to have been poisoned include wild turkeys (*Meleagris gallopavo*) that were killed by predators taking advantage of birds concentrated at a feeding station, common grackles (*Quiscalus quiscula*) that died of infection with protozoan parasites, and a wolf (*Canis lupus*) that died of canine parvovirus infection.

In our experience, everyone involved in a controversy benefits when the actual nature of the problem is identified. There are so many real environmental problems that it is important that time and effort is not wasted by mistaken assumptions about the cause of animal death or disease. The CCWHC can play an important role in controversial situations, because our diagnostic service is perceived to be an independent source of expertise, and the findings are accepted as untainted by any conflict of interest. We value this status highly, because it benefits all those concerned with the health of wildlife resources.

Disease Updates:

Atlantic Region

Yersiniosis in Snowshoe Hares - Newfoundland

In Newfoundland there is no established program for routine monitoring of snowshoe hares (*Lepus americana*). Submissions are usually because someone sees something of

interest or hears a report of a disease which prompts further submission. On October 30th, 1997 three snowshoe hares from the St. John's area were submitted to our laboratory. The individuals who had snared them were concerned about the appearance of the internal organs. Two animals showed evidence of cestode infestation, either through the presence of cysts or migratory tracts.

The cysts were identified as *Taenia pisiformis*. The third animal, from which only the liver was submitted, had evidence of bacterial hepatitis suspected to be tularemia or yersiniosis.

On November 6th, 1997 two hares were found dead near Dildo (Trinity Bay), only one of which included internal organs. The organs of the intact animal had macroscopic lesions suggestive of tularemia or yersiniosis.

In both cases because of the possibility of tularemia, level three containment facilities were necessary for culture of the bacteria. At the time, there was no level three laboratory in Newfoundland so reference laboratories in mainland Canada were contacted. It is significant to relate the difficulty in finding a suitable laboratory to assist in this work. The responses received from most laboratories were that they either didn't have level three facilities or else wouldn't do the work as it was not part of their mandate. This raises two issues of concern. First, with increasing recognition of the need for employee safety, laboratory diagnosis is becoming both more expensive and difficult to achieve. This is very justifiable, as we cannot put our employees at risk in the face of known or suspected human pathogens and we must identify ways to meet increasing biosafety costs. The second issue relates to mandates being redefined to a narrow client-based focus. Many organizations now will not accept submissions if there is not a defined paying client. In both of these cases the owners would not have been interested in paying for the laboratory work but there was a justified public interest in knowing what diseases were present and the subsequent notification of interested parties (public health officials, wildlife officials, consuming public).

In both cases, the causative organism was identified as *Yersinia pseudotuberculosis*. This bacterium and the cestodes *Taenia pisiformis* and *T. serialis* are considered to be fairly common in our wild populations with the two cestodes being seen frequently during periods when hare populations are high.

I would like to thank those laboratories that did assist in these particular examples.

Contributor: Dr. Hugh Whitney, Director, Animal Health Division, NF Dept. of Forest Resources & Agrifoods, St. John's, NF. [New diagnostic techniques for some diseases are

helping to alleviate the first problem identified by Dr. Whitney, eg., in Vol. 4(2) we reported an immunohistochemical technique for diagnosing tularemia using formalin-fixed tissue that eliminates the need for bacteriologic culturing of the bacterium].

Mass mortality of young harp seals

Every March, many harp seals (*Phoca groenlandica*) and a lesser number of hooded seals (*Cystiphora cristata*) congregate on ice floes in the Gulf of St. Lawrence to give birth. In March 1998, the amount of ice in the Gulf was considerably less than in previous years, being concentrated at the west entrance of the Northumberland Strait, between Prince Edward Island and New Brunswick. Approximately 200,000 harp seals gave birth in this area. During the first week of April, many seals were found dead along the north shore of PEI. By the end of May, a clean-up operation had counted more than 1,400 carcasses spread over several hundred kilometers of shoreline (Chuck Gallison, PEI Department of Fisheries and Environment, pers. comm.). Less than 10% of these carcasses were those of immature hooded seals ("bluebacks"); the rest were immature harp seals, about half of which still had some of their white coat ("ragged jackets"). All animals were in very good body condition, as indicated by the thickness of their blubber (in most cases, 40 mm or more). About 25% of the carcasses were completely intact, except for scavenged eyes. In all other carcasses, either the head region or the cranial third or half of the body had lost some of, or all, its skin and blubber (sculp), the edges of the remaining portions of skin being very straight. Some carcasses had a complete circumferential cut/tear of the sculp around the body, with the entire cranial portion of the sculp missing. In others, the cranial portion of the sculp was in rags, but its repositioning over the carcass showed the cuts/tears to be in a spiral shape, sometimes going almost twice around the circumference of the body. The soft tissues of the exposed portions of the carcasses had been extensively scavenged, but there was no evidence of antemortem fracture of the exposed skeleton. Detailed necropsy of seven of eight intact carcasses failed to reveal a cause of death; the eighth had an acute generalized infection by beta-hemolytic *Streptococcus* sp.

The gruesome appearance of many of the beaches, particularly the pattern of cuts/tears on the pelt of a large proportion of the carcasses, prompted some members of the public to suggest that this mortality was associated with the activity of seal hunters. However, none of 86 carcasses examined on the beach or in the lab showed evidence of crushed skull, the standard method used by sealers to kill seals. Little is known about the life history of young harp seals and hooded seals once they are weaned. They undergo a 3-5-week-long postweaning fast, during which they seem to spend much of their time hauled out on small ice floes (Garry Stenson and Mike Hammill, Department of Fisheries and Oceans, pers. comm.). It would be reasonable to assume that the swimming skills of these young animals are not yet well developed and that their stamina is limited. The week preceding this mass mortality saw heavy storm activity in the Gulf resulting in massive compaction of ice floes (André Maillet, Canadian Coast Guard, pers. comm.). This could have forced the young seals into the water or trapped them beneath the ice. Drowning should therefore be considered as a plausible explanation for this event. Drowning in seals is typically of the dry type, i.e. is not associated with aspiration of water, and, therefore, leaves no conspicuous gross or microscopic changes.

We ascribed cuts/tears on the pelts to the activity of scavengers, but the patterns of these cuts/tears deserve more scrutiny because of their alleged relationship with the seal hunt. Similar cuts/tears commonly are seen on freshly dead seals washing up on shore at Sable Island, Nova Scotia. These mortalities are attributed to attacks by sharks, particularly Greenland sharks (*Somniosus microcephalus*), a cold-water species. Animals of this species, which can be predators as well as scavengers, are thought to tear the sculp from seals, and we verified on several carcasses that tears along the skin (but not the blubber) of these seals have indeed very sharp borders, as if they had been made by a knife. The Greenland shark is known from the northern Gulf of St. Lawrence, mainly through by-catches (Dave Gillis, PEI Department of Fisheries and Environment, pers. comm.). It is not known from the southern Gulf, but perhaps the lack of fishing activity during winter has prevented recognition of its occurrence in this region when the water is sufficiently cold for the species.

In conclusion, the exact cause of this mass mortality of young harp and hooded seals was not determined. Trauma, emaciation and infectious disease were ruled out, and drowning as a result of storm activities was considered a plausible explanation. The circumferential and spiral cuts/tears on many of the carcasses were ascribed to scavenging activity, but the details of such activity deserve more studies. (Contributors: P-Y Daoust, CCWHC, Atlantic region; Zoe Lucas, Sable Island, Nova Scotia)

Salmonellosis in passerine birds

The Atlantic provinces experienced a widespread outbreak of salmonellosis in passerine birds around bird feeders this past winter. The disease was identified at each of the four Atlantic Veterinary Diagnostic Laboratories. The common redpoll (*Carduelis flammea*) was, by far, the species most commonly affected; other species included pine siskins (*C. pinus*), evening grosbeaks (*Coccothraustes vespertinus*), and purple finches (*Carpodacus purpureus*). One case was diagnosed in a pine siskin from the Annapolis valley of Nova Scotia in early December 1997. Starting in early February 1998, cases were diagnosed in several regions of Nova Scotia (including the Annapolis valley and Halifax) and southeastern New Brunswick (including Moncton, Fredericton, and Sussex). Reports of mortality were also received from Cape Breton Island, Nova Scotia, and from northeastern New Brunswick. Interestingly, no report was received from the western half of New Brunswick, and only two cases of salmonellosis were identified in Prince Edward Island. The first reports of salmonellosis in southern Labrador were confirmed in late April, presumably as common redpolls were making their way back north. Mortality was still occurring in this region in late May, and some cases were also diagnosed in purple finches and pine siskins from Nova Scotia, New Brunswick and PEI in late May.

In a large proportion of affected birds, gross lesions were characterized by extensive necrosis of the crop and/or esophagus and by hemorrhage within the air spaces of the skull, the latter attributed to impairment of blood coagulation caused by septicemia. Many affected birds were in poor body condition, with complete absence of fat reserves and at least some degree of atrophy of the breast muscles. With very few exceptions, all isolates of *Salmonella* species from passerine birds during this outbreak were identified as *S. typhimurium*, serotype 4,5:i:2, phage type 40 (Dr. C. Poppe, Health of Animals Laboratory, Agriculture Canada, Guelph, Ontario). Isolates from various locations were susceptible to most common antimicrobials. (Contributors:

Dan Busby, CWS, Sackville, New Brunswick; Hugh Whitney, Animal Health Division, St. John's, Newfoundland; James Goltz, Provincial Veterinary Laboratory, Fredericton, New Brunswick; Lyn Ferns, Veterinary Pathology Laboratory, Truro, Nova Scotia; P-Y Daoust and Scott McBurney, CCWHC, Atlantic region)

[The outbreak reported above was part of a very widespread occurrence of salmonellosis this past winter that involved songbirds throughout the eastern and mid-western USA, and in all provinces at least as far west as Riding Mountain National Park, Manitoba. In most winters, small outbreaks of salmonellosis occur among house sparrows (*Passer domesticus*) about backyard feeders; 1997-98 was remarkable because of the geographic extent of the outbreak and the involvement of other species, particularly the common redpoll. The factors that resulted in this occurrence are unknown].

Quebec Region

Canine Parvovirus Type 2 Infection in a Wild Wolf

A one-year-old non-pregnant female wolf (*Canis lupus*) in good body condition was found dead in March 1998 in the Senneterre area, Abitibi-Temiscamingue, Quebec. The hair around the anus was coated by a small amount of haemorrhagic feces. At necropsy, the small intestinal serosa was segmentally roughened and reddened. The intestinal lumen contained a moderate amount of haemorrhagic feces. Histologically, there was a severe diffuse necrosis of intestinal crypts. The crypts were strongly immunofluorescent when stained by the use of an anti-canine parvovirus type 2 (CPV 2) antibody. There were no other significant findings.

Canine parvovirus type 2 is a well known cause of enteritis in domestic dogs. Antibodies to this virus are prevalent in wolf populations throughout North America (J Wildl Dis 30: 270-273) and this virus could adversely affect some wolf populations by causing high mortalities in pups (J Wildl Mgmt 59: 565-570). This case is the second in North America, the previous case having been recently described in Minnesota (J Wildl Dis 33: 321-322). These two cases indicate that infection of adult wolves by CPV-2 can result in acute fatal enteritis. Furthermore, these two cases occurred in winter. At this season, wolves must cover large distances for food foraging, which may result in the transmission of pathogens among packs, and the transmission of pathogens to animals that were not immunised. (Contributors: Igor Mikaelian, CCCSF; Nicole Blanchette (MEF, Rouyn Noranda), et Daniel Martineau (CCCSF).

Presumptive Atoxoplasmosis in Common Grackles

At the end of August 1997, approximately 20 common grackles (*Quiscalus quiscula*) were found dead within 48 hr around a night roostery that was used by several hundred birds. Two birds were submitted for necropsy. They both had moderate multifocal necrotizing hepatitis with a few intralesional tachyzoites. These organisms did not stain with immunohistochemistry using an anti- *Toxoplasma gondii* antibody. These parasites were putatively identified as belonging to the genus *Atoxoplasma*. Toxicologic and bacteriologic examination of the major organs of the two birds were unremarkable.

Atoxoplasma spp. is a coccidian parasite of passerine birds. The asexual portion of the life cycle occurs in mononucleated cells of the blood

(lymphocytes and plasma cells) and in the lamina propria of the intestine. The sexual portion of the cycle occurs in epithelial cells of the intestine and results in the excretion of sporulated oocysts. The infection is generally fatal in canaries and the characteristic lesions consist of necrotizing hepatitis and splenitis (Vet Clin Pathol 25: 140-141). Necrotizing pneumonia also has been

occasionally described (J Wildl Dis 32: 130-132). The disease has rarely been described in wild passerine birds (Can J Zool 49: 1105-1110) and, to our knowledge, the present report is the first description of spontaneous atoxoplasmosis in common grackles. (Contributors: Igor Mikaelian (CCCSF), J. P. Dubey (Parasite Biology and Epidemiology Laboratory, USDA, Beltsville, MD), Daniel Martineau (CCCSF).

Ontario Region

Evidence of *Leptospira* infection in raccoons from Middlesex and Kent counties, Ontario

Leptospirosis is a bacterial disease that is spread to humans mainly through the urine of infected wild and domesticated animals. Three dogs in the area of Guelph , ON were found to be positive for leptospirosis in the fall of 1996. One infection was confirmed to be *L. grippotyphosa* and the other two were possibly infected with a related strain of *Leptospira*. An additional three dogs from the same area were positive for *L. grippotyphosa* in the fall of 1997 (Dr. John Prescott, Ontario Veterinary College, pers. comm.). In November 1997, the Middlesex-London Health Unit in London, Ontario investigated four trappers with clinical symptoms compatible with leptospirosis and *L. grippotyphosa* was confirmed in three of the trappers. Transmission of leptospiral organisms from raccoons *(Procyon lotor)* was suspected because all four trappers had captured and handled raccoons from Middlesex county in the weeks prior to their infections. To more clearly define the prevalence and distribution of *L. grippotyphosa* in raccoons from Middlesex and Kent counties, animals were live-trapped in these two counties from 25 April to 2 May, 1998. Serum was collected from 36 adult (15 & 21) raccoons captured within five localities in southwestern Middlesex county (i.e., townships of Metacalfe, Mosa, Ekfrid, & Caradoc) and 6 adult (4 & 2) raccoons captured in Rondeau provincial park in Kent county. Serological testing using the microscopic agglutination test revealed that 10 of the 42 raccoons had antibody titres 1:50 against *L. grippotyphosa* (range, 1:50 to 1:200). Seropositive raccoons were observed in each of the six localities tested with an overall seroprevalence to *L. grippotyphosa* of 23.8% (range, 20 to 40%). Leptospiral infection in raccoons from southern Ontario appears to be widespread. **Trappers and other individuals who frequently collect raccoons should wear rubber or latex gloves when handling or skinning these animals to minimize direct skin contact with urine and other body fluids.** (Contributors -Bryna Warshawsky L. Middlesex-London Health Unit, 50 King Street, London, ON, N6A 5L7; Robbin Lindsay, & Harvey Artsob, Federal Laboratories of Health Canada, 1015 Arlington Street, Winnipeg, MB, R3E 3P6)..

Toxicity Events

A red fox (*Vulpes fulva)* found dead in February on a railway line near Bancroft had 45 ppm of strychnine in its stomach contents. The contents did not look like material such as meat or dogfood whicih might commonly be used as bait. Nonetheless, the investigating officer suspected malicious poisoning and had requested testing for strychnine. Strychnine acts on the central nervous system to produce tetanic convulsions and rigidity of extensor muscles. It has been in use as an animal poison for centuries. In the USA, its use is restricted to below ground applications against rodents. (Contributors: Doug Campbell, CCWHC; Rick Dodd, OMNR) Bancroft).

In November, 1997, five Canada geese (*Branta canadensis*) were found dead adjacent to a small lake in an industrial park in Brampton. At approximately the same time the previous year, 18 geese were found dead at the same site. No diagnosis was reached in that case. In both instances, gross and microscopic examination revealed primarily pulmonary congestion and edema and congestion of viscera. Animals from the 1996 episode tested negative for organophosphate and carbamate pesticides, strychnine and for all toxic compounds by mass spectrophotometry-gas chromatography. Animals from the 1997 event also tested negative for organophosphates, carbamates and strychnine. Samples of liver were submitted to the Toxicology Laboratory at Michigan State Universitiy where zinc phosphide was identified by gas chromatography. Zinc phosphide is used as a rodenticide, most commonly in outdoor applications, such as protection of orchards and ornamental trees from voles. Late fall applications are common. The geese presumably contacted the bait while grazine on grass adjacent to the lake. (Contributors: Caroline Brojer, CCWHC; Andrew Taylor, CWS; Wilson Rumbeiha, MSU Toxicology Lab.)

In March, 1998 a mature bald eagle from Elgin County, near Lake Erie was found to be lethargic and weak and was brought to a veterinary clinic in Woodstock, where it subsequently died. At necropsy, the eagle was in fair body condition. There were multifocal areas of pallor in the myocardium, suggestive of myocardial necrosis, and there was marked pulmonary congestion and edema. Microscopically, there were areas of myocardial necrosis and fibrosis, associated with degenerative changes in cardiac blood vessels. Other arteries were similarly affected. There was cerebral edema and degenerative changes in several peripheral nerves examined. Tissue lead levels were markedly elevated: 44 ppm in liver and 18 ppm in kidney. Mercury levels in kidney were 160 ppm dry weight, a level that has been associated with pathological effects in raptors. Low (9.5 ppm dw) liver levels and elevated kidney levels suggest chronic exposure to mercury. No source of lead was identified. (Contributors: Doug Campbell, CCWHC; Pud Hunter, OMNR Aylmer).

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