An outbreak of West Nile virus-associated disease in domestic geese (*Anser anser domesticus*) upon initial introduction to a geographic region, with evidence of bird to bird transmission

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Abstract — West Nile virus activity in Manitoba was documented for the first time by the collection of an infected crow found on July 8, 2002, in Winnipeg. West Nile virus was identified as the cause of death for a large number of domestic geese at a single farm in southern Manitoba in August. Of the 5 differently aged cohorts on the affected farm, which included 2 breeding flocks and 3 growing flocks, the 6-week-old cohort was most severely affected with 692 of 2731 goslings dying within a 10-day period. Seroprevalence of West Nile virus in 2 clinically affected and recovered juvenile cohorts was 98% and 100%. In breeding geese without clinical disease, seroprevalence was 90% for 15-month-old birds and 10% for 5-year-old birds. Seroreaction in 3 of 4 cohorts tested exceeded what would be expected by mosquito transmission alone.

Résumé — Flambée de la maladie causée par le virus du Nil occidental chez l'oie domestique (*Anser anser domesticus*) lors de sa première apparition dans une nouvelle région géographique, avec indication de transmission d'oiseau à oiseau. L'apparition au Manitoba du virus du Nil occidental a été rapportée pour la première fois à la suite de la récupération d'une corneille infectée le 8 juillet 2002 à Winnipeg. En août, le virus du Nil occidental a été reconnu comme étant la cause de la mort d'un grand nombre d'oies domestiques d'une même ferme du sud du Manitoba. Des 5 cohortes d'âges différents de la ferme affectée, dont 2 troupeaux reproducteurs et 3 d'engraissement, la cohorte âgée de 6 semaines a été le plus durement touchée : 692 des 2731 oisons sont morts en 10 jours. La séroprévalence du virus du Nil occidental dans les 2 autres troupeaux d'engraissement affectés, mais qui se sont rétablis, était de 98 et 100 %. Chez les oies de reproduction sans signes cliniques, la séroprévalence était de 90 % pour les oiseaux de 15 mois et 10 % chez ceux de 5 ans. La séroréaction chez 3 des 4 cohortes testées était plus intense qu'attendue pour une transmission par moustiques uniquement.

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Introduction

West Nile virus (WNV) belongs to the family *Flaviviridae* in the Japanese encephalitis serocomplex group and is transmitted by adults of various species of *Culex* mosquitoes, primarily among birds, but occasionally to humans and other mammals, where clinical disease may occur (1). In late summer of 1999, the first domestically acquired human cases of West Nile encephalitis were documented in the United States (2). It is not known how the New York 1999 strain of WNV was introduced into the United States, but it is most closely related genetically to strains found in the Middle East, primarily in Israel in 1998 (3,4).

West Nile virus was first confirmed in Manitoba on July 15, 2002, from tissue from a crow found dead on July 8 in Winnipeg. Mosquito and avian surveillance in Manitoba prior to 2002 did not detect WNV in the region. Areas of the United States adjoining Manitoba were also considered free of WNV prior to the summer of 2002 (5).

West Nile virus was identified as the cause of death for a large number of domestic geese at a single farm in southern Manitoba in August. Of the 5 differently aged cohorts on the affected farm, which included 2 breeding flocks and 3 growing flocks, the 6-week-old cohort was most severely affected, with 692 of 2731 goslings either dying or being euthanized for humane reasons within a 10-day period.

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The purpose of this paper is to report on the first incident in North America of WNV causing disease in commercial geese.

Clinical diagnostic submissions

On August 15, 2002, 3, 4-week-old Embden geese (1 live, 2 dead), and 2, 17-week-old geese (1 live, 1 dead) were submitted to the provincial veterinary laboratory for necropsy. The owner reported that the sick birds appeared "dizzy" and that 6 deaths had occurred in the last few days. The live birds appeared depressed and somewhat ataxic, with drooping necks and heads that could be raised by hand with no resistance and would slowly sink back to the drooped position upon release. This clinical picture of low numbers of young geese affected by a syndrome of sudden death, severe depression, weakness, and ataxia has previously been associated with erysipelas bacteremia and septicaemia, in Manitoba and elsewhere (6).

Complete necropsies were performed. No obvious gross lesions were evident in the older birds. Slight pale streaks were present in the myocardium of 1 of the younger birds. No other significant findings were evident. Tissues were fixed in 10% neutral buffered formalin and then routinely processed and stained with hematoxylin and eosin for histopathologic examination. Tissues, including those from lung, spleen, liver, and brain were submitted for routine bacteriologic examination. Samples of brain and kidney were submitted for reverse transcription-polymerase chain reaction (RT-PCR) examination for WNV. Tissues were homogenized and RNA was extracted using the QIAamp viral RNA kit (Qiagen, Mississauga, Ontario). Real time and standard one-step RT-PCR were performed using primer and probe sets directed against the envelope, NS3, and 3' non-coding regions of the WNV genome (7).

In the younger birds, 1 of 3 had a moderately severe, nonsuppurative myocarditis, characterized by interstitial lymphoplasmacytic infiltrates, multifocal necrosis and loss of myofibers, and focal areas of early myocardial fibrosis. One of the younger birds had nonsuppurative encephalitis with moderate perivascular lymphocytic cuffs, focal gliosis, scattered neuronal degeneration, and satellitosis. Lesions were present in the cerebrum, midbrain, brain stem, and cerebellum. Mild nonsuppurative meningitis was also evident. In this bird, severe lesions were also present in the cerebellar folia and consisted of multifocal poliomalacia with infiltration of macrophages, capillary prominence, necrosis and loss of Purkinje cells, focal gliosis, and edema of the Purkinje cell layer. In 2 of 3 younger geese, mild focal gliosis and mild nonsuppurative meningitis were the only lesions. Two of the younger geese had acute multifocal splenic necrosis and lymphoid depletion, 1 of 3 had acute multifocal pancreatic necrosis and periductal lymphocytic infiltrates, and l of 3 had a few scattered foci of acute coagulative hepatic necrosis.

In 1 of the 17-week-old geese, nonsuppurative encephalitis and severe cerebellar lesions, as described in the younger geese were present. In the 2nd older bird, mild focal gliosis was evident in the brain stem. Other lesions in both birds included multifocal splenic necrosis and lymphoid depletion in 1, multifocal pancreatic necrosis and interstitial lymphocytic infiltrates in 1, prominent periportal lymphocytic infiltrates in both, and scattered lymphocytic infiltrates in the kidneys in both. Morphologic diagnoses on the various birds included nonsuppurative meningoencephalitis, nonsuppurative myocarditis, multifocal necrotizing splenitis with lymphoid depletion, and multifocal necrotizing pancreatitis.

No significant bacterial isolates were obtained from the lung, spleen, liver, or brain of the younger birds. A moderate to heavy growth of *Escherichia coli* was obtained from the spleen, liver, and brain of 1 of the older birds. Brains and kidneys from both 4-week-old birds and the 17-week-old birds were positive for the WNV genome by RT-PCR.

Upon request of the senior pathologist (JG Spearman), 6 more 5-week-old dead birds were submitted for postmortem on August 19. The predominant lesions identified in these submissions were nonsuppurative encephalitis and myocarditis. Pooled brain and kidney from 2 birds were positive for the WNV genome by RT-PCR in all 3 specimen sets.

On August 23, subsequent to a significant increase in the number of acutely ill birds on the farm, 6 live goslings, now 6 wk old, were submitted for postmortem examination. At the time of submission, the owner reported having lost 150 individuals in this age cohort to sudden death and another 450 had been destroyed for welfare reasons, as they were moribund. This new clinical picture was compatible with highly pathogenic avian influenza or exotic Newcastle disease, both of which are reportable diseases in Canada. An independent investigation by the Canadian Food Inspection Agency on August 26 did not identify either of these reportable diseases of poultry as the cause of mortality (Dr. Andre Dore, personal communication, September 9, 2002). The farm was not quarantined.

Histologic lesions in birds from the August 23 submission consisted predominantly of nonsuppurative myocarditis and nonsuppurative meningoencephalitis with severe cerebellar lesions, as described in the previously submitted geese. Three samples (2 birds each) of pooled brain tested positive by RT-PCR for WNV. Oral and cloacal swabs from 3 live birds tested negative for WNV antigen with a rapid immunochromatographic assay (VecTest; Medical Analysis Systems, Camarillo, California, USA).

Farm investigation

The farm was visited by 1 of the authors (RAA) on August 24 during the peak of the clinical disease outbreak. Significant morbidity and mortality was restricted to the youngest cohort of geese and was estimated to be 40%. Clinical expression in an individual was characterized by ataxia, proceeding rapidly to recumbency over 3 to 5 h. Healthy appearing geese were frequently observed pecking at the feces and open wounds of clinically affected birds. Feather picking, a behavioral problem of young geese, had been considered a problem in this flock prior to the disease outbreak. Recumbent individuals were exposed to aggressive cannibalization, if not removed into a sick pen.

A comprehensive on-farm investigation was initiated on September 5, which included a site inspection, a review of records, and detailed interview with the producer. The

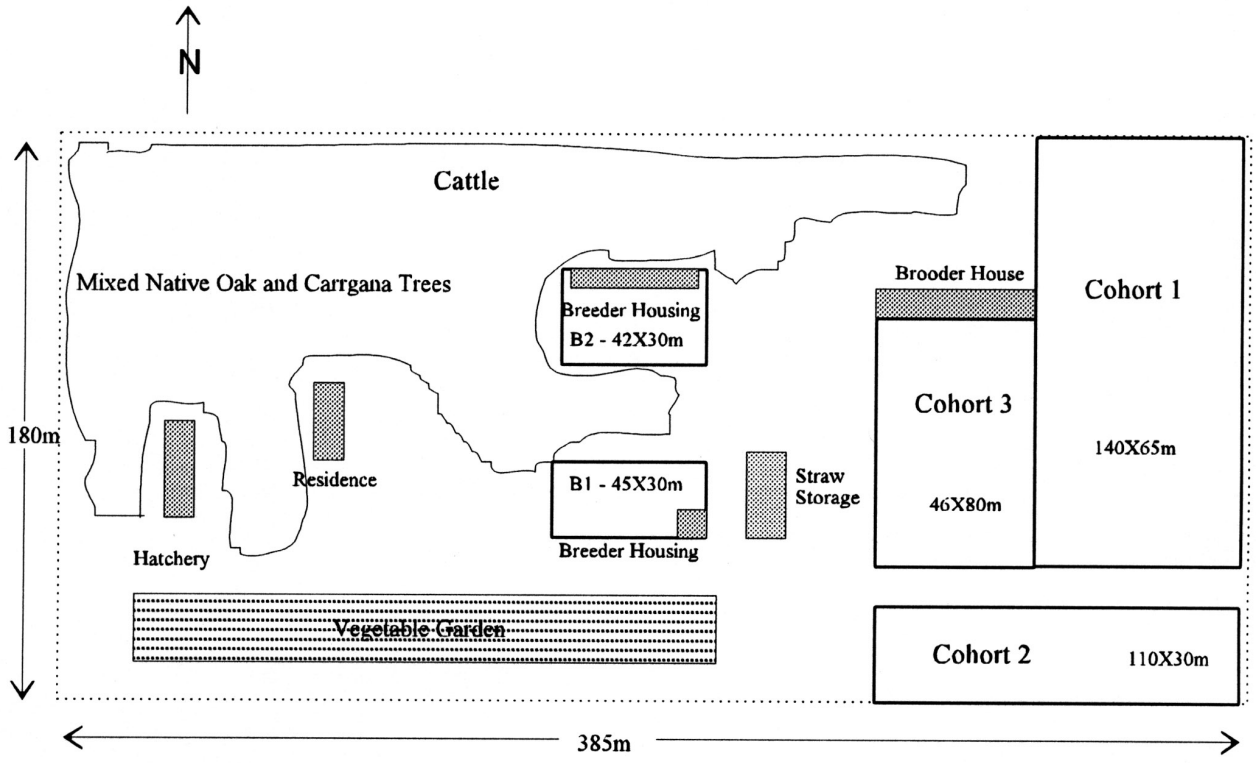

Figure 1. Topographical representation of the layout of the premises where West Nile virus (WNV) was identified in geese. Diagram is not precisely to scale and some small outbuildings are not shown. Buildings appear in shading; outdoor pens for holding growing and breeding geese appear as open rectangles. Approximate dimensions of pens are given with the cohort of birds that were present in that pen on September 5. Breeding 1 (B 1) and B 2 are the location of breeding flocks, and cohort 1, 2, and 3 are the location of the 3 growing cohorts, in order of decreasing age. This image was redrawn from an orthographical photograph taken 09/95, Manitoba Agriculture and Food, Agri-Map Services, and confirmed in consultation with the farm manager.

Table 1. Description of goose inventory

Cohort	Date of hatch	Inventory on July 20	Death loss July 20 to August 31	Fence line contact
Older breeder flock (B 1)	1998	400	0	None
Young breeder flock (B 2)	2001	750	0	None
Cohort 1 (C 1)	April 15, 2002	3717	55	C 3
Cohort 2 (C 2)	May 27, 2002	3218	125	None[a]
Cohort 3 (C 3)	July 15, 2002	2932	893[b]	C 1

[a]This group of geese was separated from the other groups by a runway 3 m wide that was used to bring feed into the pens and to move geese between pens
[b]Six hundred and ninety two birds of 2731 alive on August 20, died between August 20 to 30. Twenty additional birds from this cohort were euthanized in week ending September 7, due to failure to recover from neurologic symptoms compatible with WNV infection

farm is an integrated breeding-to-finishing goose facility. Goose production is the primary commercial activity at this location. The goose production component had been in continual operation for more than 15 y and contains a hatchery committed to water fowl, a brooding facility, and 2 separate buildings for holding breeder flocks during the winter. The livestock operation resides on a contiguous plot of about 7 ha, including the residence. An estimated 35% of this area is heavily wooded (Figure 1). The farm also contained a small hobby chicken flock of less than 25 birds; 5 unconfined Pekin ducks; a herd of beef cattle, numbering 25 in total; 2 large livestock guard dogs; and a pet sheep. Clinical disease consistent with WNV was confined to the geese. No other poultry operations were identified within 1 km of this operation.

On September 5, there were 5 age cohorts of geese on the premises held as groups (Table 1). All cohorts were of mixed sex and the Embden breed. Farm records were well maintained and death losses for each cohort had been recorded manually on a calender. Information from farm records was used to create a weekly death loss histogram (Figure 2).

Production practices were similar to those of other farms in Manitoba and included goslings that were not sold off farm as day-olds were being brooded in an enclosed, environmentally controlled facility for 4 to 7 wk. Birds from the 1st hatch were kept enclosed for longer due to

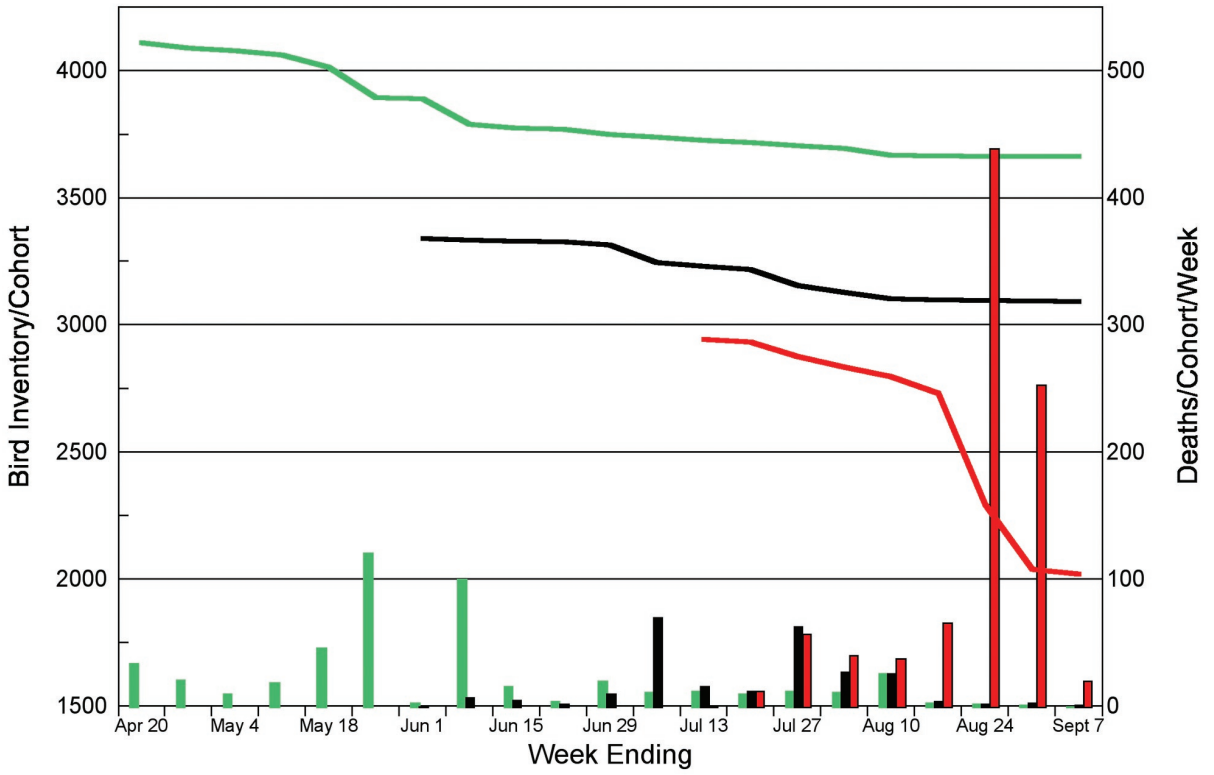

Figure 2. Time line of death loss and inventory change for the 3 growing cohorts of geese on the premises. Bars are death loss per week on the right axis, line is decreasing inventory left axis. X-axis is the chronological week of 2002. Green bar and line is cohort 1 hatched April 15, black bar and line is cohort 2 hatched May 27, and red line and bar is cohort 3 hatched July 15, 2002. Cohort 3 was not released from the barn until 3-wk of age and death loss during the week ending July 27 may not be attributable to West Nile virus (WNV) infection.

the inclement weather and cool evening temperatures that are usual during the early summer in Manitoba. For the youngest cohort retained at the end of enclosed brooding, the goslings had access to the outdoors and could return to the brooder at night. For older cohorts of growing birds, the goslings were held in outdoor pens exclusively, as the next flock requires the brooder. Outdoor pens provide at least 1 m^2/bird.

The brooding facilities at this location were designed to optimally hold 3600 goslings per hatch. During this production year, the 1st hatch (cohort 1 [C 1]) was 4144 goslings on April 15. Of these, 2444 originated from the on-site hatchery and 1700 originated from an off-site hatchery. Subsequent hatches on April 25 (2742), May 6 (3165), and May 15 (2424) were sold as day-old goslings to other producers. The May 27 hatch of 3340 goslings remained on the farm and is referred to as cohort 2 in this report. Hatches on June 7, June 14, June 22, and July 1, numbering 2216, 1925, 1467, and 1478 goslings, respectively, were sold off farm. Cohort 3, which hatched on July 15, contained 2944 day-old birds, composed of 919 birds hatched on farm and 2025 goslings moved onto the farm from another hatchery.

In this production system, there is normally a cull at hatching for anatomical and other defects, and a proactive cull for leg soundness at the time the flock is turned outside at 5 to 6 wk of age. The cull for leg soundness is shown in Figure 2 as increased death loss for C 1 on week ending May 25 and for C 2 on week ending July 6. The owner attributed the loss of 100 birds in C 1 to hypothermia, subsequent to a storm during the weekend of June 8; an additional 16 birds were lost in a storm on June 15, when the brooder house was occupied by C 2.

The C 2 flock was treated for coccidiosis in week 4 of production, while in the brooder house. The C 3 hatch displayed clinical illness with poor feed consumption and increased mortality in the 1st full week of production. At 10 to 14 d of age, this cohort was treated with antibiotics in the water (Neochlor; J. Webster Laboratories, Victoriaville, Quebec). This nonspecific disease resulted in the loss if 147 birds from this group between the July 15 hatch and the week ending August 10. The C 3 group was released from the brooder barn on August 1 without sorting or handling and with no cull for leg soundness. The owner reported increased death loss in C 2 during week ending July 27 (63 birds), continuing into the next week (25 birds). During week ending August 10, 26 birds were lost each from C 1 and from C 2, triggering the initial laboratory submission on August 15. The owner reported that it was highly unusual to lose more than 1 to 3 birds/wk in groups more than 6 wk of age. Historical production records from previous years were not available for inspection.

During the weeks ending August 24 and August 31, when the majority of birds were lost, the owner reported that the illness in C 3 was primarily characterized by severe ambulatory impairment without significant central nervous system (CNS) depression. If recumbent birds

Table 2. Results of hemagglutination-inhibition (HI) testing of goose serum for flavivirus

Cohort	Number sampled	Number positive at greater than 1:20[a]
Old breeder flock (B 1)	30	3
Young breeder flock (B 2)	40	36
Cohort 2 (C 2)	40	38
Cohort 3 (C 3)	30	30

[a]Results of initial screening test for the presence of antibody to flavivirus using a standard HI test (8)

Table 3. Serum result agreement between laboratories[a]

Cohort	Individual sample	HI endpoint titer (screening)[b]	PRNT endpoint titer WNV[c]
B 1	29	<1:10 (negative)	negative
B 1	30	1:80	1:640
B 2	15	<1:10 (negative)	negative
B 2	16	1:20	1:40
C 2	24	1:80	1:1280
C 3	29	1:160	1:320
C 3	30	1:40	1:80

HI — hemagglutination inhibition test; PRNT — plaque reduction neutralization test; WNV — West Nile virus
[a]A subset of samples was titrated to endpoint by the HI test and then tested without blinding to the previous results, with the PRNT
[b]Results of initial screening test for the presence of antibody to arbovirus using a standard HI test (8)
[c]Plaque reduction neutralization test endpoints of specific antibody to WNV (9)

were placed on their feet, they would often haltingly move to the feed and water containers and commence to eat and drink. During the acute phase (August 20 to 30), recumbent birds that could not walk after being placed on their feet were euthanized for humane reasons, as otherwise they would have died of dehydration or cannibalism. As this phase of the outbreak progressed, individual affected birds could be identified 1 to 2 d prior to the owner being able to capture them easily for segregation. The owner reported that approximately 30% of the birds placed in the sick pen would recover, as long as they retained some ability to walk. One-third of the goslings in this age group died or were euthanized due to clinical disease that was consistent with WNV-associated disease (Table 1).

During the inspection on September 5, all cohorts of geese appeared healthy and reacted normally to the presence of the veterinarian and farmer. An estimated 10% of the remaining C 3 goslings had drooping wing tips. This presentation was judged to be a non-neurological sequella to feather picking, where the absence of the major flight feathers prevents the gosling from locking the wings in the normal position on the lateral surface of the abdomen. The owner reported finding no new sick birds since September 2. It appeared that by September 5, active disease was no longer present on the farm.

Serological investigation

On September 28, blood samples were collected for diagnostic testing. Cohort 1 had been marketed at this time and was unavailable for sampling. Of the 3470 birds marketed from this cohort, only 117 did not grade as "A", which was similar to production cycles in other years.

Of the 4 cohorts remaining on the farm, C 2 was being sorted on this date to select 800 previously sexed birds for breeding purposes. Forty of those birds were selected for blood sampling. Thirty samples were collected from each of 2 groups destined for slaughter; namely, C 3 and the 5-year-old breeding flock (B 1). Forty blood samples were taken from individuals in the young breeding flock (B 2), which was to be retained for a 2nd breeding season.

Blood samples were tested for the presence of antibody to flaviviruses by using a standard hemagglutination-inhibition (HI) test incorporating suckling mouse brain WNV antigen. The testing procedure used was as previously described (8), that is, using bovine albumin borate saline (BABS) as the hemagglutination diluent, protamine sulfate treatment of the serum, and gander red blood cell suspension (G. Smart; Cadham Provincial Laboratory, Winnipeg, Manitoba).

Within the C 3 cohort, which had experienced the severe clinical signs, 100% of the birds tested were seropositive (Table 2). There was a high seropositive rate in the other growing cohort, C 2 (95%), and in the B 2 breeding flock (90%).

Due to the HI test being cross reactive with a variety of flaviviruses, representative serum samples from individuals that were HI positive and individuals that were HI negative for antibody to flavivirus were also tested for WNV-specific antibody by using a plaque reduction neutralization test (PRNT) (9). All HI positive samples tested by PRNT displayed significant titers of antibody specific to WNV antigen, while antibody specific for other flaviviruses, such as St. Louis encephalitis virus, were not detected (Table 3).

Discussion

Sampling of individuals for diagnostic examination was not random. Geese presenting for postmortem examination were selected by the producer as thought to be representative of the flock situation. For the serologic investigation in 4 cohorts, individuals were convenience selected for blood collection by herding a small group into an enclosure to facilitate manual capture and restraint. In the C 2 flock, blood samples were collected only from the subset of birds retained for future breeding. This method of selecting individuals from this cohort was chosen to minimize animal handling and to minimally interfere with other management related

activities occurring at the same time. Additional bias may have resulted in sampling this subset, as the selection of future breeding birds was based on conformation and body weight, so individuals in this subset may have been, on average, more robust than was the C 2 group as a whole.

Prior to the 2002 vector season, WNV was not present in the region, and it is reasonable to assume that seropositive samples represented recent seroconversion.

West Nile virus has been identified as an annual cause of morbidity and mortality in domestic geese in Israel from 1997–2000, primarily manifesting as a neuroparalytic disease of 3- to 8-week-old goslings (10). The Israeli strain of WNV causing goose mortality is closely related to the strain currently circulating in North America (3). In experimentally infected young domestic geese, the New York strain of WNV caused depression, weight loss, torticollis, opisthotonus, and death with accompanying encephalitis and myocarditis (11).

The evidence presented in this outbreak is consistent with the possibility that the premises was first infected sometime during the week ending July 20, resulting in a spike in mortality in C 2, which did not trigger a laboratory submission. Clinical disease on this farm appears to have been biphasic and sensitive to the age of the birds. There was an abnormal increase in death loss starting in the week ending July 27 and continuing in the subsequent 2 wk in all the growing age cohorts. This resulted in the first presentation of birds for diagnostic pathology on August 15. This limited outbreak was followed by a dramatic disease manifestation in the last 2 wk of August that was limited to the youngest cohort of growing geese.

It is unlikely that mosquito transmission alone would have resulted in the seroprevalence identified in 3 of the 4 age cohorts sampled during a single season exposure. Seroconversion of free-ranging waterfowl to WNV in endemic areas has not been well documented; however, an 8% seroprevalence rate was found in ducks in France, presumed to be subsequent to a single season of WNV exposure (12). Serological results from this current outbreak support the hypothesis that there was direct bird to bird virus transmission, even where clinical manifestations were mild or absent, as in the B 2 group. In laboratory situations, direct transmission of WNV occurred among goslings without an insect vector (11). West Nile virus has been demonstrated experimentally from cloacal swabs taken from 17 of 24 avian species and in oral secretions of 12 of 14 avian species (13); however, the antigen panel assay (VecTest) was negative on a single sampling of live geese presented for postmortem during this outbreak in Manitoba.

In the 2 asymptomatic breeding cohorts, the high level of seroprevalence in the B 2 cohort was unexpected. The B 2 seroprevalence rate found in this instance is consistent with individual birds becoming infected, developing a high virus titer, and subsequently transmitting virus directly to other in contact birds without incurring concurrent clinical disease themselves. The seroprevalence rate in the B 1 cohort is consistent with vector transmission alone; however, none of the data required to fully assess the methods of transmission could be obtained from this outbreak.

Other than a nonspecific protective effect of age, there is no obvious explanation as to why the 5-year-old breeding group did not seroconvert to a similar extent as the younger cohorts. It is unlikely that this was due to differential mosquito exposure at this location. The maximum physical separation between groups of birds on the premises was 50 m, which is well within the flight distance of mosquitos. Age specific resistance to viremia and disease caused by WNV infection is well documented for chickens (14).

Although it is fair to describe this outbreak as neuroparalytic disease, many of the clinical signs could be attributed to weakness that can be secondary to myocardial degeneration or general malaise associated with virema. In addition, gait abnormalities observed, including recumbency, could be explained equally well by CNS or peripheral nerve involvement. It is clear from this study that a wide range of clinical disease in geese is consistent with infection with WNV.

In 2001, 138 farms in Manitoba contained a total of 59 620 geese (15). In 2002, evidence of WNV was found in all parts of the province where commercial goose production is common. There were no other losses in commercial geese reported to the provincial veterinary staff during the 2002 goose production season.

Evidence from this study suggests that commercial geese are potentially an important source of WNV for mosquitoes that have the opportunity to feed on them, especially after widespread direct transmission that may quickly amplify virus in a defined population. A high infection rate in young geese, as evidenced in this case study, combined with the physical incapacity that affected many of the infected birds was such that they would have been incapable of avoiding mosquito attack. Acutely infected multi-age flocks would potentially provide a significant source of infection for mosquitoes. Investigation should be initiated into the prevalence and level of cloacal, oral, or other method of virus shedding in naturally WNV-infected farmed geese, as well as into blood titers to determine the risk of such flocks becoming peridomestic reservoirs of this virus for potential transfer to humans. Serological studies of people in Israel indicate a strong association between seropositivity to WNV and contact with the agricultural production of geese (16). The control of WNV in commercial geese housed close to people or horses may be in the public interest.

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