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Wild ducks are the reservoir for only a limited number of influenza A subtypes

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SUMMARY

Analysis of cloacal samples collected from 12321 wild ducks in Alberta, Canada, from 1976 to 1990 showed influenza A infections to be seasonal, with prevalences increasing as the population became increasingly more dense. Viruses with 3 haemagglutinin (H3, H4, and H6) and 3 neuraminidase subtypes (N2, N6, and N8) were found consistently to infect both adult and juvenile ducks each year, indicating that wild ducks may be a reservoir for these viruses. In contrast, viruses with 7 haemagglutinin (H2, H5, H7, H8, H9, H11, and H12) and 3 neuraminidase subtypes (N1, N3, and N4) were not found for prolonged periods during the study; when they were found, they primarily infected juveniles at moderate levels. Whilst wild ducks appear to perpetuate some influenza A viruses, they apparently do not act as a reservoir for all such viruses.

INTRODUCTION

Since 1889, six influenza A pandemics have occurred among humans, with the earliest documented outbreak of what was probably influenza A occurring in 1173 [1, 2]. The 1918 outbreak of H1N1 influenza resulted in 10–25 million deaths worldwide [3, 4], making pandemic influenza one of the most fatal diseases of this century.

Although the origin of influenza pandemics remains to be fully elucidated, antigenic and genetic studies have shown that the haemagglutinins (HA) and neuraminidases (NA) of the 1957 and 1968 pandemic strains are closely related to those of viruses found in ducks [5, 6]. When pigs or other mammals are coinfected with both human and avian influenza A strains, the viral gene segments may in theory reassort to form up to 256 different gene combinations. Phylogenetic

studies of influenza A viruses have revealed species-specific lineages of viral genes and demonstrated that human pandemic strains and all current mammalian influenza A viruses are derived either by direct transfer of avian influenza viruses or by genetic reassortments between human and avian viruses [7].

The occurrence of influenza A infections among wild ducks in nature is important because their annual migrations put them in contact with large numbers of other animal species that could potentially become infected. Although viruses possessing haemagglutinin H13 have been found only in shorebirds and gulls, all 13 of the other haemagglutinins and all 9 neuraminidases are represented in influenza viruses found in wild ducks [8]. Investigators have long speculated that wild ducks are the primary reservoir for influenza A viruses. Infections caused by most strains of influenza virus are asymptomatic in aquatic birds, indicating that avian viruses are highly adapted to their natural hosts [8]. In wild ducks, influenza viruses replicate preferentially in the cells lining the intestinal tract and are excreted in high concentrations in the faeces [9]. Influenza viruses have been isolated from unconcentrated lake water, indicating that waterfowl are able to transmit influenza viruses both to other ducks and to other domestic and wild birds by depositing faeces in their water supply [10].

Previously, it was not clear if wild ducks maintained all influenza A subtypes, transmitting them both to other waterbirds and to other mammals, or if they maintained particular viral strains, with other waterbirds serving as a reservoir for other influenza A viruses and, in turn, transmitting them to ducks. This study was conducted to determine if wild ducks maintained all influenza A strains over time and to determine if the prevalence of influenza A viruses in wild ducks differed according to whether or not these viruses were maintained in shorebirds or gulls. Another goal of this study was to examine and describe seasonal differences in prevalences of avian influenza. To address these questions, we measured the prevalences of all known influenza A strains over a 15-year period in a population of approximately 6 million wild ducks that breed in Alberta, Canada. We also examined prevalences of paramyxovirus infections over time in this wild duck population to determine if there were seasonal differences in these prevalences and to investigate possible associations between paramyxovirus and influenza A virus infections.

MATERIALS AND METHODS

Sample collection

From 1976 to 1990, Canadian Wildlife Service personnel annually sampled ducks in Alberta, Canada, at the end of the duck breeding season when ducks were assembling at lakes to begin their migration south. As shown in Table 1, ducks were sampled at lakes near Vermilion from 1976 to 1978, near Grande Prairie from 1979 to 1984, and near Edmonton from 1985 to 1990. While most samples were collected during August, samples were also collected in late July and early September. In 1984, a subset of samples was collected in April when ducks were nesting (Table 1).

Canadian Wildlife Service personnel caught ducks in cloverleaf traps baited

Table 1. *Migratory ducks banded, cloacal sampled and tested for infections with paramyxoviruses and influenza A viruses, 1976–90, Alberta, Canada*

Year	Sampling location	Number of ducks banded	Collected samples		Processed samples	
			Number	Percentage	Number	Percentage
1976	Vermilion	4006	946	23·6	943	99·7
1977	Vermilion	4622	2566	55·5	2436	94·9
1978	Vermilion	5160	2251	43·6	1865	82·8
1979	Grande Prairie	3685	1486	40·3	1316	88·6
1980	Grande Prairie	2104	738	35·1	735	99·6
1981	Grande Prairie	3291	788	23·9	754	95·7
1982	Grande Prairie	2697	818	30·3	818	100·0
1983	Grande Prairie	4108	808	19·7	806	99·8
1984	Grande Prairie	3120	1172*	37·6	1138	97·1
1985	Edmonton	3850	904	23·5	901	99·7
1986	Edmonton	4979	901	18·1	100	11·1
1987	Edmonton	7032	308	4·4	97	31·5
1988	Edmonton	8531	211	2·5	204	96·7
1989	Edmonton	6660	200	3·0	82	41·0
1990	Edmonton	6047	295	4·9	126	42·7
Total		69892	14392	20·6	12321	85·6

* 348 of the 1984 samples were collected in April when ducks were nesting.

with grain. Cloverleaf trapping of ducks does not select a random sample of the duck population at each site because more aggressive birds such as mallards and adult males of all species are more likely to gain access to the grain and thus be caught. However, cloverleaf traps were used during all 15 years of the study, allowing year-to-year comparisons. Wildlife personnel banded ducks and recorded their species, sex, and age, using a process described in more detail elsewhere [11]. Age categories were limited to juveniles, defined as ducks born within the year of sample collection, and adults, defined as birds one or more years old. A total of 69892 ducks were banded during the 15-year period of the study (Table 1).

Wildlife Service personnel collected cloacal samples from 14392 ducks, or 20·6% of the 69892 ducks banded (Table 1). In selecting ducks to be sampled for the study, workers gave priority to mallards (*Anas platyrhynchos*), and they attempted to sample ducks throughout each banding period. Although samples were more likely to be collected when extra workers were available and weather conditions favourable, we attempted to sample ducks without systematic bias. As shown in Table 1, the percentage of banded ducks from which cloacal samples were taken varied over the 15-year period of the study, peaking at 55·5% in 1977. Mallards were the most frequently sampled species, comprising 62·8% of samples; of ducks sampled, 19·8% were pintails (*An. acuta*), 11·8% were blue-winged teals (*An. discors*), and 5·7% belonged to 1 of 11 other duck species.

Unweighted band return data for migrating mallards banded in Alberta show that 46% use the Pacific Flyway, 29% the Central Flyway, and 25% the Mississippi Flyway; Alberta mallards infrequently use the Atlantic and Mexico Flyways [12]. Mallards banded at the Grande Prairie and Edmonton sites primarily use the Pacific Flyway. Of mallards banded at Vermilion, approximately 33% use the Central Flyway, 19% the Pacific Flyway, and 47% the Mississippi

Flyway. Pintails sampled at all three sites predominantly use the Pacific Flyway [12].

Virus identification

Over the 15-year period of the study, we processed 12321 of the 14392 cloacal samples collected (85·6%) (Table 1). All samples were placed at −70 °C upon arrival at St Jude Children's Research Hospital, and frozen samples were taken randomly for processing. Viruses were grown in the allantoic cavity of 11-day-old embryonated chicken eggs [13]. Haemagglutinin titrations and haemagglutinin-inhibition tests were performed in microtitre plates with receptor-destroying, enzyme-treated sera [14]. Neuraminidase titrations and neuraminidase-inhibition tests were done as described by Aymard-Henry and colleagues [15]. All haemagglutinating agents were identified in inhibition tests with specific antisera to the isolated surface antigens of reference influenza viruses or avian paramyxoviruses [13].

Epidemiologic analysis

To make seasonal comparisons of prevalences of paramyxovirus and influenza A virus infections, we calculated the mean prevalences of infections with influenza A viruses and paramyxoviruses according to week of sample collection. We then calculated mean prevalences for each of the calendar weeks in late summer when samples were collected. We restricted analysis to weeks when at least 10 adult or juvenile samples were collected, and we did not calculate mean prevalences for a calendar week unless samples were collected during that calendar week in at least three seasons. Because infections with influenza A viruses are known to be more prevalent among juveniles than adults [13], prevalences were calculated separately for each age group. In 5 of the 15 years, for example, 10 or more adult samples were collected during the first week of August; in 8 of the study years, 10 or more juvenile samples were collected that same week. We calculated prevalences for each of these weeks separately; we then calculated mean prevalences of both viruses for that calendar week for adults and juveniles. Similarly, we calculated mean prevalences for ducks sampled during the second, third, fourth, and fifth weeks of August and during the first week of September.

We combined samples according to their year of collection to make comparisons of prevalences across the 15-year period of the study. Because the prevalence of influenza A infections differed according to calendar week of sample collection, we did not calculate prevalences for years when sample collection did not extend over at least 3 weeks during August (i.e. 1976 and 1990). In 1980, adults were sampled only the last week of August, while juveniles were sampled over a 5-week period; thus adult prevalences for this year were excluded from analysis. For 1984, calculated prevalences excluded samples collected in April of that year.

To determine if sex and age characteristics of each year's duck population affected infection prevalences, we analysed data on the Alberta wild duck population supplied by the Canadian Wildlife Service. One parameter used was the percentage of ducks banded each year that the Service classified as juvenile. We also used banding data to determine annual estimates of the percentage of banded adult ducks that were male. A second parameter was the age structure of

the duck population based on examination of harvested ducks [16]. Each year the Service asks a random sample of hunters to return a wing from each duck killed. Because the plumage of adults and juveniles differs, Service personnel are able to determine age of harvested ducks by analyzing these wings. We used the harvested wing data to calculate the percentage of the duck population that was juvenile for each year of the study.

We compared our parameters of the age and sex make-up of the population with yearly prevalences of paramyxovirus and influenza A infections. We dichotomized age and sex measures at the mid-points of their distributions, calculated mean prevalences of infection at each level, and used t tests and two-tailed P-values to compare the means. We also used multiple linear regression to predict influenza A infection prevalences based both on undichotomized measures of the age and sex composition of the population and on prevalences of paramyxovirus infection. To examine changes in prevalence over time, we grouped influenza strains together according to haemagglutinin subtype only and calculated yearly prevalences for each of the 12 haemagglutinins found in Alberta ducks. We similarly grouped strains together according to neuraminidase subtypes and calculated yearly prevalences for each of the nine neuraminidases. We plotted these prevalences over the study period, again excluding years when samples were collected for fewer than 3 weeks in August and excluding the 1984 spring samples.

RESULTS

Seasonality

Fig. 1 shows the relationship between week of summer sample collection and mean prevalence of influenza A and paramyxovirus infections. Patterns of infection with influenza A viruses were seasonal for both adults and juveniles, with prevalences consistently higher for juveniles than adults regardless of week of sample collection. Among adults, influenza A prevalences were low at the beginning of August, peaked in mid-August, and declined during the last week in August. Among juveniles, influenza A prevalences were relatively low at the beginning of August but then increased and remained elevated through the first week of September. Among adults, mean prevalences of paramyxovirus remained consistently low throughout August. Among juveniles, mean paramyxovirus prevalences were consistently higher than for adults; juvenile prevalences remained fairly constant through the end of August and then dropped in September.

Only 1 of 92 juveniles sampled during April 1984 was infected with influenza A virus (H11N9), a prevalence of 11 per 1000 juveniles for this time period. None of the 208 adult ducks sampled in April 1984 was positive for influenza A virus.

Population age and sex make-up v. *infection prevalences*

Table 2 describes the relationship between the age and sex composition of the population and the prevalence of influenza A and paramyxovirus infections over the study period. Measures of the age structure of the population shown here are the percentages of banded and harvested ducks that were juvenile. Among both adults and juveniles, average prevalences of paramyxovirus infection were higher

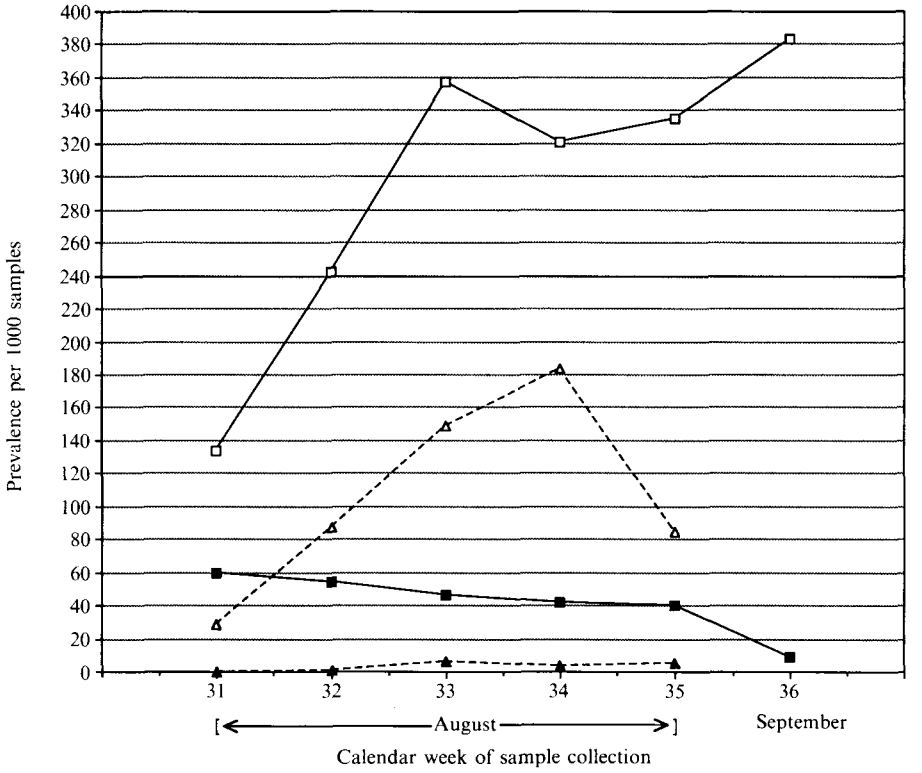


Fig. 1. Seasonal trends in prevalence of influenza A virus and paramyxovirus in wild ducks, late summer samples, Alberta, Canada, 1976-90. Juvenile mean prevalences for each calendar week were based on the following numbers of samples collected over the following numbers of years: week 31, 1067 samples, 8 years; week 32, 2603 samples, 13 years; week 33, 1481 samples, 11 years; week 34, 1517 samples, 11 years; week 35, 644 samples, 7 years; and week 36, 67 samples, 3 years. Mean prevalences for adults were based on the following numbers of samples collected over the following numbers of years: week 31, 484 samples, 5 years; week 32, 1823 samples, 10 years; week 33, 795 samples, 9 years; week 34, 692 samples, 7 years; and week 35, 416 samples, 6 years. (□, Influenza A virus in juveniles; ■, paramyxovirus in juveniles; △, influenza A virus in adults; ▲, paramyoxovirus in adults.)

during years when a lower percentage of the Alberta duck population was juvenile, but these differences were not statistically significant. Results were similar regardless of the measure used to assess the age structure of the population.

In comparison, regression analysis showed average prevalences of influenza A infection among both adults and juveniles to be higher when a greater proportion of the population was juvenile (Table 2). Among adults, average influenza A prevalences were 68·3 per 1000 when harvest data showed 35–60% of the population to be juvenile *v.* 174·4 per 1000 when a higher percentage was juvenile (regression $P = 0{\cdot}08$). Among juveniles, average influenza A prevalences were 227·6 per 1000 and 367·3 per 1000, respectively, when a lower or higher percentage of the population was juvenile (regression $P = 0{\cdot}06$). Results were similar when banding data were used to assess the age structure of the population. Average paramyxovirus and influenza A virus prevalences among both adults and

Table 2. *Yearly prevalence per 1000 cloacal samples of paramyxovirus and influenza A virus infections according to age and sex composition of the population. Late summer samples, 1976–90, Alberta, Canada**

Population composition	Prevalence, paramyxovirus		Prevalence, influenza A virus	
	Adults (Mean ± S.E.)	Juveniles (Mean ± S.E.)	Adults (Mean ± S.E.)	Juveniles (Mean ± S.E.)
Age: banded ducks, juvenile (%)				
Low (10–34%)	8·4 ± 6·7	47·2 ± 15·7	92·6 ± 37·8	265·3 ± 49·6
High (35–80%)	3·9 ± 1·4	42·6 ± 8·5	150·1 ± 36·5	315·1 ± 65·8
t-test *P*	0·53	0·79	0·30	0·57
Regression *P*	0·75	0·66	0·02	0·10
Age: harvested ducks, juvenile (%)				
Low (35–60%)	9·6 ± 6·5	55·4 ± 13·2	68·3 ± 26·0	227·6 ± 40·6
High (64–72%)	2·7 ± 1·3	32·2 ± 7·1	174·4 ± 35·9	367·3 ± 66·1
t-test *P*	0·34	0·17	0·04	0·09
Regression *P*	0·63	0·62	0·08	0·06
Sex: banded adults, male (%)				
Low (56–69%)	4·8 ± 1·5	50·8 ± 14·3	151·8 ± 48·0	319·7 ± 80·2
High (70–80%)	7·1 ± 5·8	39·5 ± 9·7	99·7 ± 30·4	268·4 ± 38·4
t-test *P*	0·71	0·52	0·36	0·56
Regression *P*	0·56	0·48	0·97	0·78

* Analysis limited to summers when ducks were sampled 3 or more weeks in August. Juvenile and adult samples from 1976 and 1990 and adult samples from 1980 were excluded. In all, 6966 juvenile samples and 3748 adult samples were included in this analysis.

juveniles were not significantly related to the sex composition of the adult population.

Paramyxovirus v. *influenza A virus prevalences*

To determine if there was a relationship between prevalences of paramyxovirus and influenza A virus in the wild duck population, we compared their seasonal prevalences using linear regression (Table 3). Because of the significant relationship between influenza prevalence and age composition of the population, we included the percentages of harvested ducks that were juvenile in regression models. Controlling for differences in the age structure of the population, we found average influenza A prevalences among juveniles were positively related to average paramyxovirus prevalences among adults ($\beta = 6{\cdot}1$; $P = 0{\cdot}06$) and negatively related to average paramyxovirus prevalences among juveniles ($\beta = -3{\cdot}6$; $P = 0{\cdot}01$). Average influenza A prevalences among adults were also positively related to average paramyxovirus prevalences among adults and negatively related to average paramyxovirus prevalences among juveniles, but these relationships were not statistically significant. Of the 324 avian paramyxoviruses identified in this study, 93% were type 1.

Table 3. *Multiple linear regression coefficients (β) from models predicting yearly prevalence of influenza A infections in wild ducks per 1000 samples. Late summer samples, 1976–90, Alberta, Canada**

	Influenza A, adults ($n = 12$, $R^2 = 46{\cdot}7\%$)		Influenza A, juveniles ($n = 13$, $R^2 = 67{\cdot}9\%$)	
Independent variable	$\beta \pm$ S.E.	P value	$\beta \pm$ S.E.	P value
Intercept	$-152{\cdot}8 \pm 153{\cdot}1$	0·35	$-27{\cdot}0 \pm 88{\cdot}4$	0·89
Paramyxovirus prevalence, adults	$3{\cdot}8 \pm 2{\cdot}5$	0·16	$6{\cdot}1 \pm 2{\cdot}9$	0·06
Paramyxovirus prevalence, juveniles	$-1{\cdot}3 \pm 1{\cdot}0$	0·22	$-3{\cdot}6 \pm 1{\cdot}1$	0·01
Population, juvenile† (%)	$5{\cdot}1 \pm 2{\cdot}4$	0·07	$7{\cdot}5 \pm 3{\cdot}0$	0·03

* Analysis limited to summers when ducks were sampled 3 or more weeks in August. Adult samples were excluded in 1976, 1980, and 1990. Juvenile samples were excluded in 1976 and 1990. In all, 6966 juvenile samples and 3748 adult samples were included in this analysis.

† Based on wing analysis of harvest sample of ducks.

Prevalences over the study period

As shown in Fig. 2, prevalences of paramyxovirus and influenza A infections were consistently higher for juveniles than for adults from 1977 to 1989. Paramyxovirus infection prevalences among adults were fairly constant over the time span, while paramyxovirus prevalences for juveniles showed more year-to-year fluctuation. Influenza A virus prevalences for both juveniles and adults fluctuated a great deal from year to year, with infection rates invariably higher for juveniles than for adults.

The 12 haemagglutinins and 9 neuraminidases found in Alberta ducks generally occurred either consistently or sporadically over the timespan of the study. Fig. 3 depicts adults and juvenile prevalences of 3 haemagglutinins (H3, H4, and H6) and 3 neuraminidases (N2, N6, and N8) that followed the consistent pattern. Prevalences of these viruses were moderately high in both adults and juveniles for most years of the study, in some instances reaching epidemic levels in juveniles. For example, H6, N2, and N6 viruses infected more than 250 of 1000 juveniles sampled during some years. Prevalences of some of these six consistent virus subtypes followed a 2-year cycle, peaking one year, declining slightly the second, and declining greatly thereafter. For example, H4 viruses in juveniles peaked in 1978 at 93 per 1000 and then declined to 76 per 1000 in 1979 and 11 per 1000 in 1980. All the virus subtypes included in Fig. 3 followed this 2-year pattern to some extent, but the pattern was especially clear in some instances, such as H6 patterns in 1978–9 and 1985–6 and N8 patterns in 1979–80.

As illustrated in Fig. 4, prevalences of 10 virus subtypes (H2, H5, H7, H8, H9, H11, H12, N1, N3, and N4) followed a sporadic pattern. Typically these influenza A subtypes were not found for prolonged periods; H5 viruses, for example, were not found from 1977 to 1987. When these sporadic subtypes did occur, they primarily infected juveniles but at moderate levels, with prevalences rarely exceeding 10–20 cases per 1000 juveniles and never exceeding 50 per 1000 juveniles.

Influenza A viruses for which prevalences could not be clearly classified as either

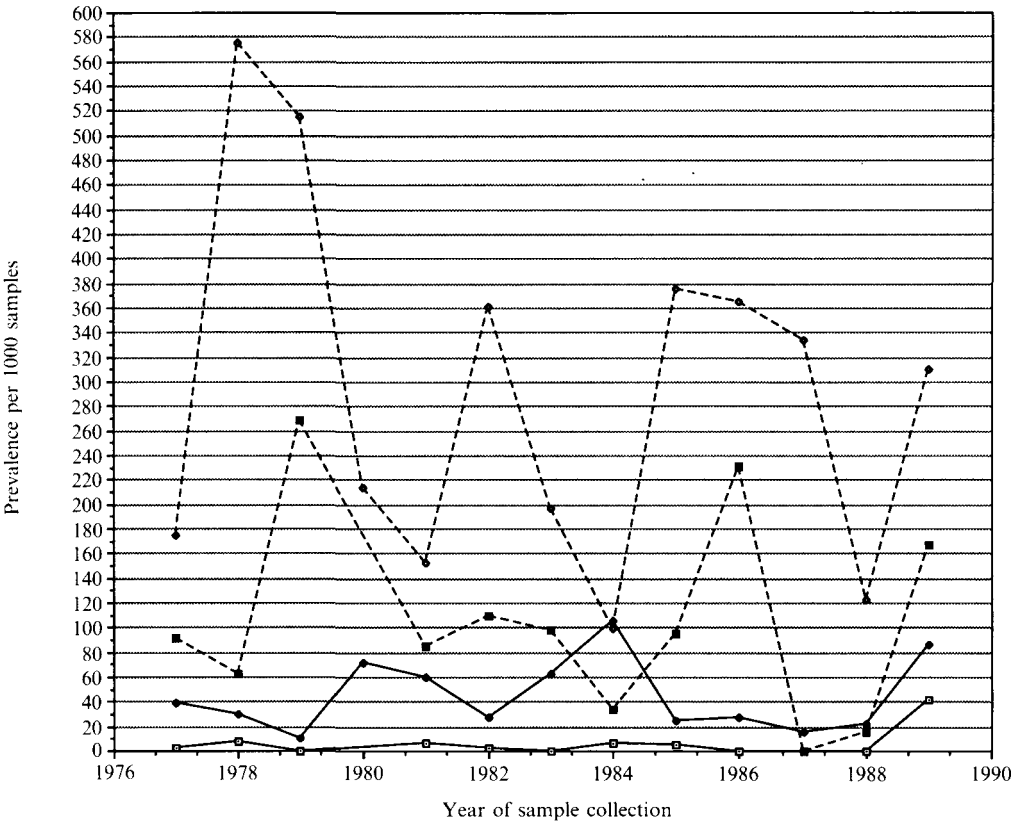
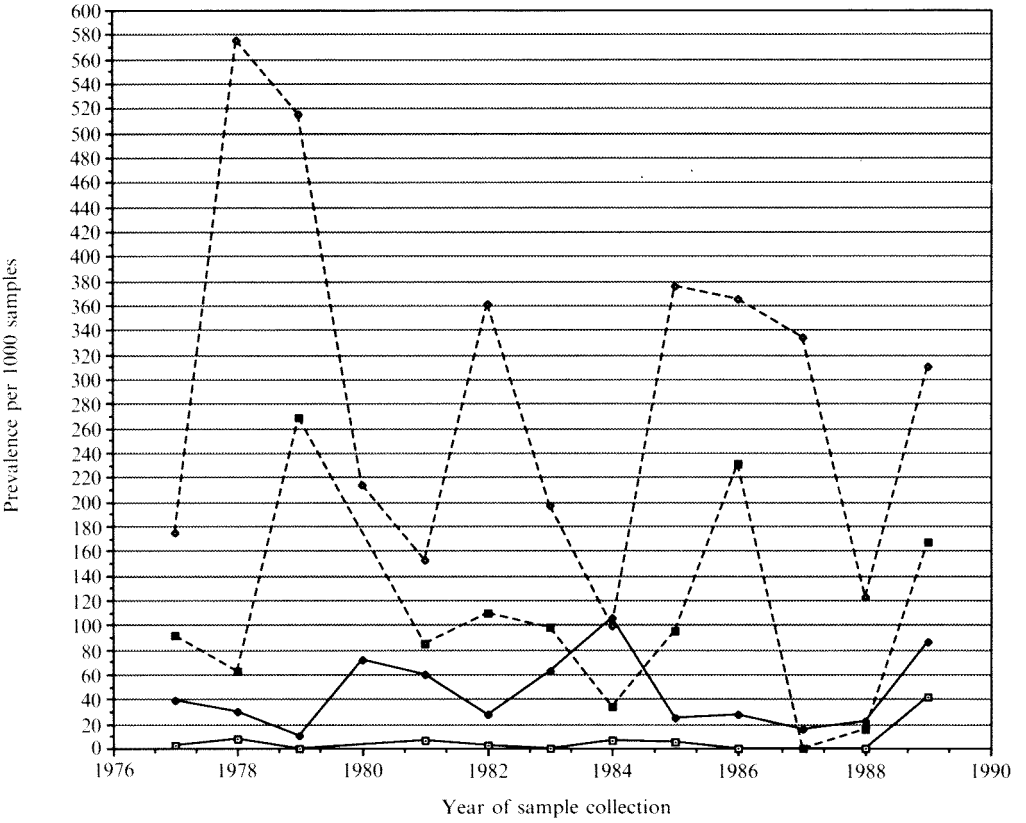


Fig. 2. Yearly prevalences of influenza A virus and paramyxovirus infections for juvenile and adult ducks from 1976–90. Analysis was restricted to years when at least 10 adults or juveniles were sampled each week during at least 3 weeks of the summer. (◇. Influenza A virus in juveniles; ◆. paramyxovirus in juveniles; □. influenza A virus in adults; ⊡. paramyxovirus in adults.)

consistent or sporadic are illustrated in Fig. 5. Although H1, H10, N5, and N7 viruses primarily infected juveniles at moderate levels, prevalences in excess of 100 per 1000 ducks were found. Although prevalences of N9 viruses never exceeded 14 per 1000 juveniles, these viruses consistently infected both juveniles and adults throughout the study.

Influenza A viruses that frequently infect shorebirds and gulls (H4, N6, H9, H11, and N9) [17] are marked with asterisks in Figures 3, 4, and 5. H13, an influenza A virus that frequently infects shorebirds and gulls, was not found in the Alberta duck population. Prevalence patterns for H4 and N6 viruses were consistent; these were moderately high in both adults and juveniles throughout the study period (Fig. 4). N6 infections were especially high in 1982 when prevalences reached 339 per 1000 in juveniles and 98 per 1000 in adults (Fig. 3). Prevalences of shorebird viruses H9, H11, and H13 either followed the sporadic pattern in ducks or, in the case of H13, were not found at all (Fig. 4). Outbreaks of H9 and H11 viruses in ducks occurred infrequently and almost exclusively in juveniles, with periods up to 6 years when these viruses were not found. Outbreaks



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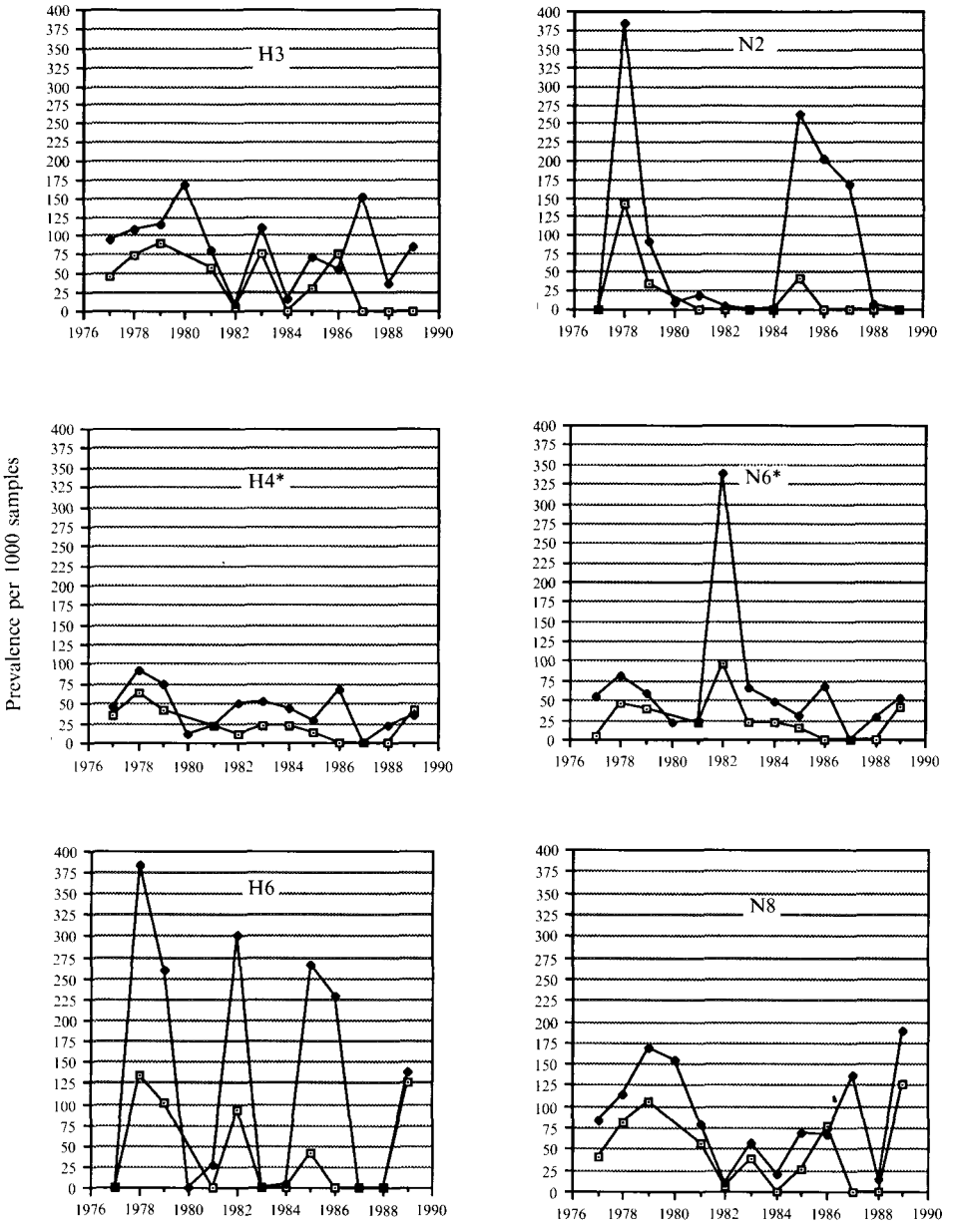

Fig. 3. Prevalences of haemagglutinin (HA) and neuraminidase (NA) subtypes that exhibited a consistent pattern. These subtypes infected both adults and juveniles consistently throughout the study period, occasionally at high levels. (* HA and NA subtypes frequently infecting shorebirds and gulls; ◆, juveniles; ⊡, adults.)

that did occur in juveniles were moderate, with prevalences never exceeding 14 per 1000 ducks (Fig. 4). Prevalences of N9 viruses, which also frequently infect shorebirds and gulls, could not be clearly classified as following either the sporadic or consistent pattern (Fig. 5).

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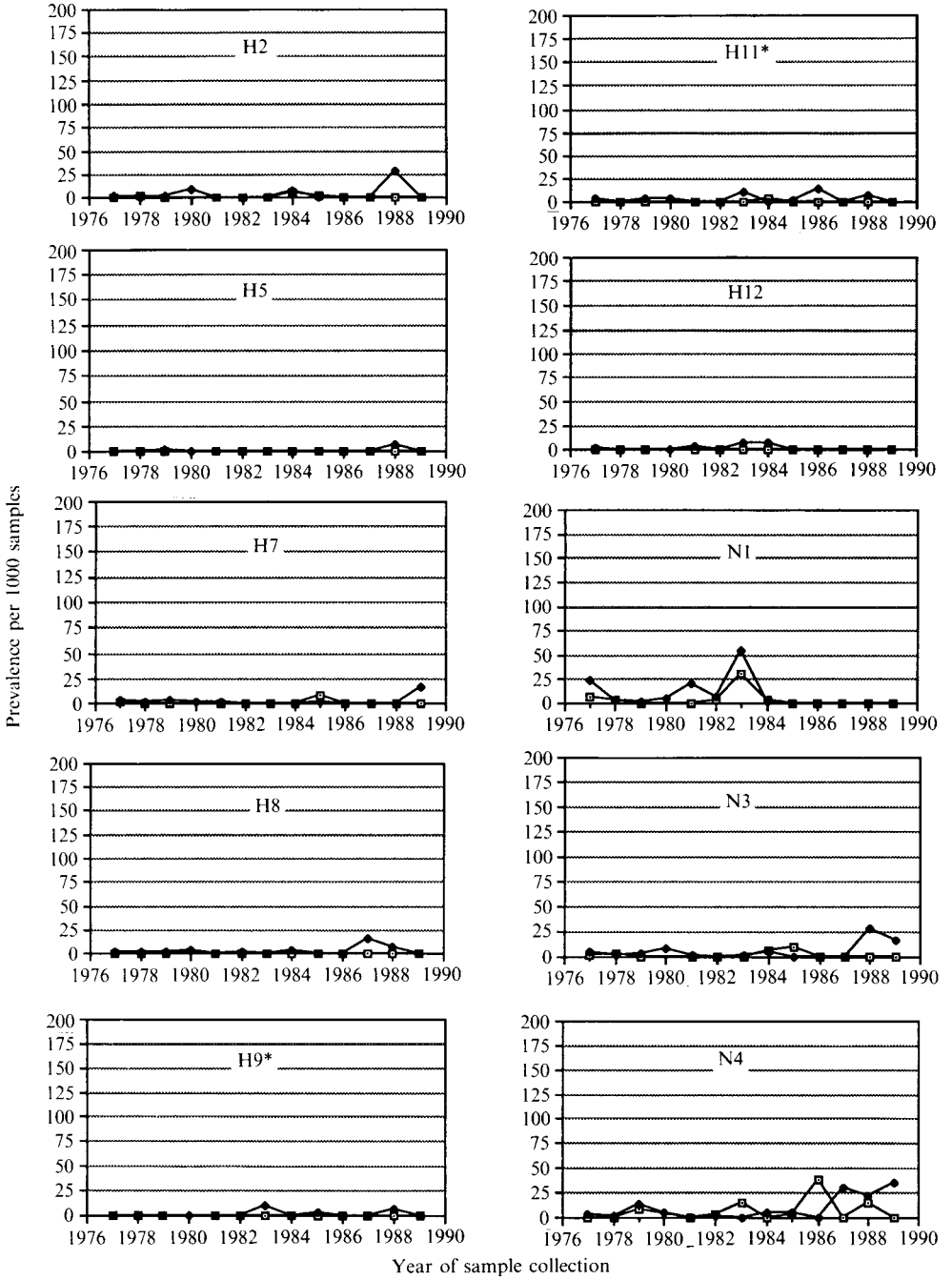

Fig. 4. Prevalences of haemagglutinin (HA) and neuraminidase (NA) subtypes that exhibited the sporadic pattern. These subtypes did not infect either adults or juveniles for prolonged periods during the study. Infections that did occur were in juveniles at moderate levels. (* HA and NA subtypes frequently infecting shorebirds and gulls: ◆, juveniles; ⊡, adults.)

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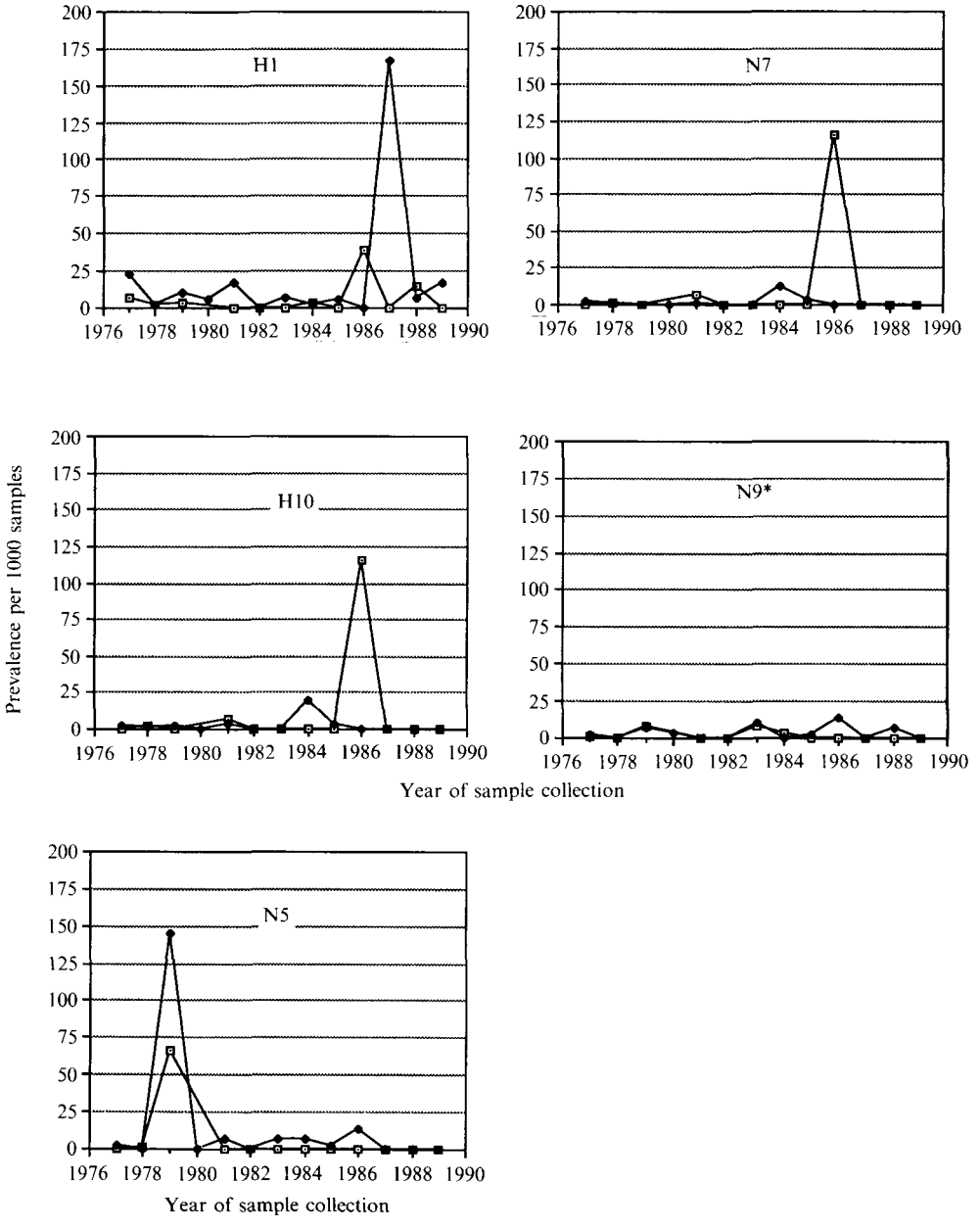

Fig. 5. Prevalences of haemagglutinin (HA) and neuraminidase (NA) subtypes that did not exhibit either the consistent or sporadic pattern. (* HA and NA subtypes frequently infecting shorebirds and gulls; ◆, juveniles; ⊡, adults.)

DISCUSSION

Our study shows that juvenile ducks are more vulnerable than adult ducks to paramyxovirus and influenza A virus infections. On almost every occasion studied, prevalences were higher among juveniles than adults. When prevalences were plotted separately for each of the 12 haemagglutinins and 9 neuraminidases

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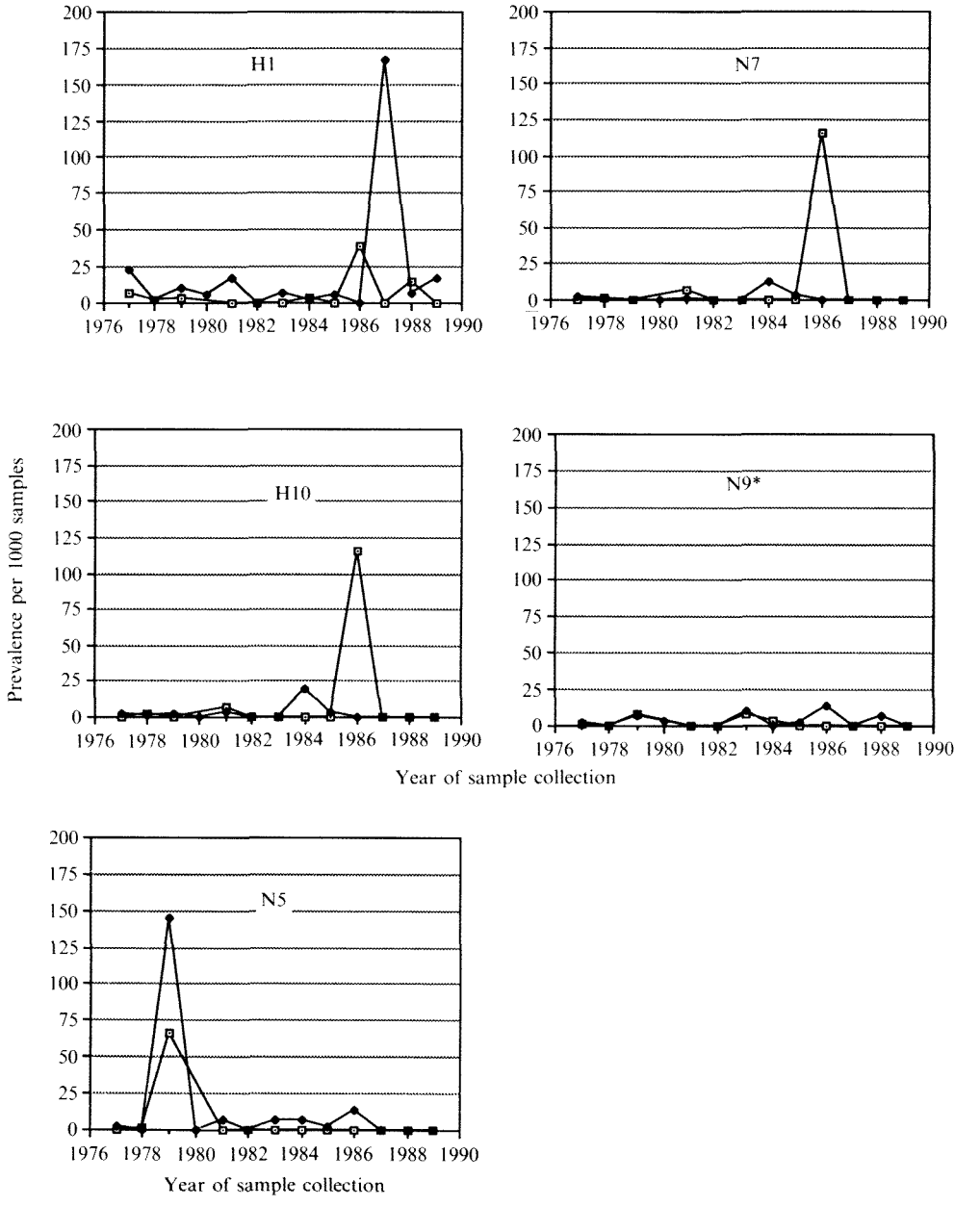

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found in Alberta ducks, they were also consistently higher for juveniles than adults. Laboratory studies have shown that ducks exhibit limited antibody response after initial influenza infection and can be reinfected with the same virus 46 days after primary inoculation, suggesting a limited antibody immunity to influenza viruses [18]. However, the cell-mediated immune response of ducks may not be short-lived and, if not, might explain why influenza A virus infections are more prevalent among juveniles than adults. When a larger portion of the duck population is juvenile, the number of infected ducks per lake is greater because influenza A virus prevalences are higher in juveniles than adults. Thus, our finding that adult and juvenile prevalences are significantly higher when a larger proportion of the population is juvenile could be the result of increased virus concentrations in lakes and ponds at such times.

Measures of age and sex composition of the duck population used in this study are somewhat biased. Because juvenile and female ducks are less likely to force their way into grain-baited traps than the more aggressive adult males, banding data underestimate both juveniles and females. On the other hand, since juveniles are more vulnerable to hunters than adults, harvest data overestimate juvenile numbers. Both banding and harvest measures, however, are likely to be strongly correlated with true levels of juveniles and males in the population, and since the same sampling techniques were used throughout the study, these measures should be comparable over time. Although wildlife biologists consider the harvest measure a better measure of the age structure of a duck population, our results were similar regardless of the measure used.

The fact that three sampling areas were studied consecutively and not in parallel must be considered in interpreting our results. Although ducks from the three areas exhibit somewhat different migration patterns, the sampling sites are in close enough proximity that large percentages of ducks at each site use the same wintering grounds. For example, the percentage of mallards using the Pacific Flyway was similar for ducks banded at the Grande Prairie and Edmonton sites, where samples were collected for 12 of the 15 years of the study. No relationships between changes in sampling sites and viral prevalences were apparent. Although collecting samples at one location throughout the study would have been preferable, it seems unlikely that changes in sampling location had a major effect on our results.

Our results show that paramyxovirus and influenza A prevalences among juveniles are inversely correlated at statistically significant levels. In an earlier study of eggs infected with both influenza A viruses and paramyxoviruses, influenza A viruses were preferentially detected over paramyxoviruses [19], and this might account for our finding that paramyxovirus and influenza A virus prevalences are inversely related. Alternatively, the relationship might be due to viral interference in infected birds or differences in immunologic defences.

The prevalence of influenza A virus was seasonal in Alberta ducks, being extremely low in April, moderate at the beginning of August, peaking in mid- and late August, and remaining elevated in juveniles through the first week of September although declining in adults in late August. This cycle correlates with the grouping together of ducks at staging areas before they begin their southern migrations. The prevalence rose as increasing numbers of ducks gathered at the

lakes where we collected samples, and both duck numbers and influenza prevalences peaked in late August and September when as many as 50000 ducks may have been present per lake. Thus, our finding of seasonality in the prevalence of influenza A infections may reflect increased viral contamination of lakes and ponds resulting from the increased duck population. Since many of the ducks gathering at these migration staging areas are separated during nesting and breeding periods, this mixing together of ducks should maximize the number of different influenza viruses present in lakes and ponds at this time. Thus, it is reasonable for prevalences of specific influenza subtypes and of influenza A infections in general to be higher at this time.

Our study shows that prevalences of particular haemagglutinins and neuraminidases fall into two major types of patterns. Subtypes H3, H4, H6, N2, N6, and N8 were consistently found in both adults and juveniles year after year, with prevalences occasionally reaching moderately and even extremely high levels. In a prior study [20], these same viral subtypes were also frequently found circulating among New York ducks. The consistent presence of these subtypes in both adult and juvenile ducks suggests that wild ducks serve as a reservoir for these viruses. Since H6 and N6 viruses are also frequently found in shorebirds and gulls, they may also serve as reservoirs.

Wild ducks do not appear to provide a reservoir for all influenza viruses. The sporadic prevalence patterns for H2, H5, H7, H8, H9, H11, H12, N1, N3, and N5 viruses support a conclusion that wild ducks do not serve as a reservoir for these influenza A viruses. Even though we sampled ducks at a time of year when the density of the duck population and presence of juveniles made it likely that influenza infections were at their annual peak, these subtypes were not found for extremely long periods of time. When these haemagglutinins and neuraminidases did appear in the population, they tended to infect only a small percentage of juveniles and virtually no adults. H13 and H14 viruses were not found at all in this wild duck population. The patterns of infection for these 10 haemagglutinins and neuraminidases imply that animals other than ducks serve as reservoirs for these viruses and sporadically transmit them to wild ducks.

Our results are consistent with the findings of Kawaoka and colleagues who reported that only half the shorebird viruses they attempted to transmit to ducks were able to replicate [17]. They concluded that at least a portion of the gene pool of influenza viruses in shorebirds and gulls is different from that in ducks and that this portion of the gene pool is maintained in shorebirds and gulls [17]. Ruddy turnstones (*Arenaria interpres*) and sanderlings (*C. alba*), two species of shorebirds known to be infected frequently with influenza viruses, migrate to breeding grounds in the Arctic on paths that intersect those of wild ducks. Thus, one might speculate that shorebirds carry influenza viruses north with them and pass them on to juvenile ducks. Our present data and previous findings [17] support these conclusions: H9, H11, H13, and N9 viruses are maintained in shorebirds and gulls: H3, H6, N2, and N8 viruses are maintained in wild ducks; and ducks, shorebirds, and gulls jointly serve as a reservoir for H4 and N6 viruses. Other waterbirds, as yet unidentified, may serve as reservoirs for the eight other viruses not frequently found in ducks, shorebirds, or gulls: H2, H5, H7, H8, H12, N1, N3, and N4. It is not clear if ducks serve as reservoirs for H1, H10, N5 and N7 viruses.

An important but long-unanswered question concerning influenza viruses in ducks is how viruses that appear to be maintained in the duck population are carried over from year to year. Influenza prevalences were extremely low during the 1984 spring nesting period, when only one of 92 juveniles and none of 208 adults sampled was infected. The single virus that was isolated was H11N9, an influenza virus with haemagglutinin and neuraminidase subtypes we found to be maintained sporadically in the Alberta wild duck population. Since H11 and N9 viruses are frequently found in shorebirds and gulls, it seems more reasonable to speculate that the single virus isolated at springtime was transmitted to ducks by shorebirds or gulls than that this virus was carried north by wild ducks during the 1984 spring migration. While our 1984 spring study provides no evidence that any influenza A viruses are maintained in ducks throughout the year and carried back north with them, this study of 92 juveniles and 208 adults might have been too small to detect low levels of influenza A viruses circulating in the population. The work of Stallkneckt and colleagues provides some evidence that influenza viruses are maintained year round in ducks [21]. Those investigators sampled both resident ducks in Louisiana and migrating ducks returning there in the fall, recovering all common haemagglutinin and neuraminidase viral subtypes from them [21]. Influenza prevalences among migrating ducks ranged from 3·1% in September to 0·4% in December and January, and influenza viruses were transmitted to resident ducks [21].

Some other overwintering mechanism might exist to allow influenza A viruses to survive both the period each year when juvenile ducks have matured and the population is primarily adult and the period when the duck population is not densely congregated. One such mechanism might be consecutive infection of ducks in the northern and southern hemispheres, since one group is breeding while the other group migrates toward warmer weather. Wild ducks may have interrelationships with, as yet, unidentified birds similar to the relationships they appear to have with shorebirds and gulls in maintaining H4 and N6 viruses. Such speculative relationships could allow H3, H6, and N8 viruses to reinfect the wild duck population year after year.

Since influenza A viruses remain infectious after longterm frozen storage, another possible overwintering mechanism might be the freezing of virus-laden faeces at staging lakes before ducks migrate south with thawing and reinfection occurring in the spring. Wildlife biologists have observed as many as 20000 ducks remaining on frozen staging lakes, churning the water to keep it open. Generally, large groups of ducks remain at staging lakes until the first snowfall eliminates their food supply and forces their migration south. When the duck population returns the next spring, many of these lakes are still frozen and must once again be churned. Thus, it is possible that viruses deposited on the ice and frozen in the fall when influenza prevalences are at their annual peaks reinfect the duck population once it returns during the spring thaw.

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