

Aquatic Birds: An Evolutionary Repository of Ortho- and Paramyxoviruses?

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INTRODUCTION

Much of the information on the ecology of avian orthomyxoviruses (influenza viruses) and paramyxoviruses (e.g. Newcastle disease virus) is recent. The recognition that the pandemic influenza virus that emerged through Hong Kong in 1968 (Chang, 1969) was antigenically related to a virus that had been isolated some years previously from a domestic duck (Laver and Webster, 1973) led to virus surveillance studies of avian and other species that commenced around 1974. These were initially fostered by the World Health Organization to see if there is a finite number of influenza viruses in nature, to gain insight into the ecology of these viruses and to see if it is possible to isolate a pandemic virus in advance of its appearance in man.

Until these studies, the only viruses from these two families about which a modest amount of information was known were those that caused fowl plague, equine and swine influenza and Newcastle disease (fowl pest). Surveillance unearthed a vast range of influenza viruses in avian species notably the domestic duck and extended the number of known paramyxoviruses.

The impetus to study lower vertebrates in the same way just does not exist. Most work has been on those of economic importance such as fish which are intensively raised under artificial conditions. Attempts to isolate viruses from reptiles have arisen mainly from sporadic attempts in the 60's and 70's to explain the ecology of arboviruses. More recently, perhaps stemming from an increased awareness of environmental issues and the need to know

more about diseases in the animal world, reptiles have been the subject of some virological studies (see Jacobson, 1986).

This report takes account of the orthomyxoviruses and paramyxoviruses of birds, their successful association with aquatic birds and attempts to evaluate in an evolutionary sense the role that reptiles may have played in handing down these viruses.

THE VIRUSES

Orthomyxoviridae

There are three types of influenza virus A, B and C based on the antigenicity of the internal ribonucleoprotein. Viruses that infect birds are all type A. The surface of the influenza virus is covered with two kinds of spiked glycoprotein projections responsible for subtype specificity. One is the haemagglutinin (H) so called because it agglutinates erythrocytes, the other is the neuraminidase (N) an enzyme that frees the virus after it has adsorbed onto a cell. A virus is subtyped on the basis of these H and N antigens. The RNA of influenza A viruses is composed of eight discrete segments, two of which code for H and N, so that in dual infection of a susceptible cell, the 16 segments may reassort to give rise to a possible 254 combinations of RNA.

The surveillance studies undertaken since 1974 have extended the known number of H subtypes to 13 which are now designated H1 to H13, and N subtypes to 9 now designated N1 to N9. Thus, influenza A viruses are subtyped H1N1, H1N2, H1N9; H2N1, H2N9, and so on.

Paramyxoviridae

There are three genera of Paramyxoviridae. The viruses that infect birds belong to the genus Paramyxovirus, Newcastle disease virus (NDV) being the prototype virus. In contrast to influenza A

viruses, its RNA is not segmented and it therefore cannot reassort. NDV also has two kinds of spiked projections one of which jointly carries both H and N activities. The second projection is responsible for haemolytic activity. Surveillance has extended the number of avian paramyxoviruses to 9, these now being designated avian-PMV1 to avian-PMV9 with NDV as avian PMV-1.

AVIAN HOSTS

Influenza

Influenza Isolations have been made from most of the major families of wild birds - Corvidae, Fringillidae, Ploceidae, Motacillidae, Scolopacidae, Columbidae, Sturnidae, Laridae, Phasianidae, Hirundinidae, Charadriidae, Ardeidae, Procellariidae, Rallidae, Alcidae, Accipitridae, Phalacrocoracidae (Lvov, 1978; Lvov and Zhdanov, 1983; Hinshaw and Webster, 1982). However, by far the majority of isolations have been from waterfowl of the family Anatidae (Table 1).

In the case of domestic and game poultry, the duck has provided the bulk of the isolates. The most extensive study on this has been in longitudinal surveillance over the period 1975-80 on domestic poultry of Hong Kong and southern China; that study amplified the importance of the duck as an influenza host (Table 2) (Shortridge 1982a and b). All the then known H subtypes, H1 to H12, apart from H8, were recorded amongst the isolates. Serological studies indicated the presence of the H8 subtype (Shortridge, unpublished data) and Shen (1983) subsequently confirmed its presence by isolation. The most common H subtypes were H4, H3 and H6 with isolation rates of 29%, 25% and 22%, respectively. All isolations were made from apparently healthy poultry. In the case of ducks, isolations were made all year round but were generally more frequent in the warmer, summer months.

Table 1. A GUIDE TO THE AVIAN HOST RANGE OF INFLUENZA A VIRUSES

Wild birds

Corvidae, Fringillidae, Ploceidae, Motacillidae, Scolopacidae, Columbidae, Sturnidae, Laridae, Phasianidae, Hirundinidae, Charadriidae, Ardeidae, Procellariidae, Rallidae, Alcidae, Accipitridae, Phalacrocoracidae and Anatidae (waterfowl especially migratory birds)

Domestic birds

Anatidae (domestic duck); other major poultry: chicken, turkey, goose
Minor poultry: pheasant, quail, partridge, Muscovy duck, mallard, Guinea fowl

Table 2. SITE OF ISOLATION OF INFLUENZA A VIRUSES FROM DOMESTIC POULTRY AT A HONG KONG DRESSING PLANT, NOVEMBER 1975 - OCTOBER 1980 (Shortridge, 1983)

Type of poultry	No. of swabs	Number	Virus isolations	
			Percent	Ratio
Duck	8737 ^a	564	6.5	16
Goose	1353	15	1.1	3
Chicken	1708	7	0.4	1

^a combined totals of cloacal and tracheal swabs

The overall isolation rate from ducks was 6.5% (Table 2) being about twice as high from the cloaca over the trachea (data not shown). The importance of water is exemplified by the isolation rates from ducks, geese and chickens which were 16:3:1, respectively. This suggested that infection is mainly intestinal and waterborne (Markwell and Shortridge, 1982). Intestinal multiplication has been demonstrated experimentally (Kida et al., 1980; Webster et al., 1980; Higgins et al., 1987). The domestic duck is intensively raised on flooded rice fields and ponds in southern China providing an ideal opportunity for continuity of influenza viruses (Shortridge, 1983) through faecally contaminated water (Markwell and Shortridge, 1982).

Migratory water birds are the other major host for influenza viruses but isolations are mainly made when these birds are assembled on lakes and bays prior to and during migrations (Hannoun and Devaux, 1980; Hinshaw et al., 1980). Very high isolation rates may be made from the birds and lake water at this time. This is not surprising as the birds are living on or near what is, in effect, a virus containing, faecally contaminated soup (Hinshaw et al., 1979; Markwell and Shortridge, 1982). It is of interest that migratory birds probably help to sustain the influenza gene pool in southern China as they pass through the region between the two hemispheres (Shortridge, 1983).

Outbreaks of disease in domestic poultry attributable to or associated with influenza viruses occur mainly in turkeys and chickens (Alexander, 1982a; APHIS, 1984; 1983; Bahl et al., 1979; Lang, 1982; Pomeroy, 1982). The ability of influenza viruses to cause disease was shown by Lang (1982) who recorded 66 outbreaks in turkeys in Ontario, Canada over the period 1960 to 1980. Similar observations have been made in California and Minnesota, USA. It is generally felt that these outbreaks are the result of introduction of virus by wild birds (Lipkind, 1982). The fact that many outbreaks in turkeys occur on farms that lie on migratory bird routes (Bahl et al., 1979; Halvorson, et al., 1985; Pomeroy, 1982) suggest introductions of virus from an outside source.

A similar situation prevails with chickens but infections have been fewer in recent years since much of the large scale raising nowadays takes place indoors. The strict separation of chickens (and turkeys) from wild and feral birds seems essential (Lang, 1982).

The main point to emerge is that the domestic duck of southern China is the major host or reservoir of influenza viruses; studies elsewhere highlight the importance of influenza viruses in causing disease in non-aquatic domestic and other birds.

Paramyxoviruses

The most important avian paramyxovirus is Newcastle disease virus (NDV), now designated as avian PMV-1. This virus comprises an antigenically related group of pathotypes which can produce a complete spectrum of virulence for most species of birds ranging from inapparent infection to 100% mortality. Symptoms include varying degrees of respiratory and neurological signs and enteritis. Other avian PMVs cause mild symptoms or none at all (Alexander, 1980).

NDV is important economically as a cause of morbidity and mortality in chickens and is the number one disease of poultry; birds must be vaccinated in those areas where the disease is endemic.

In studies on avian PMVs conducted in parallel with influenza surveillance on domestic poultry of southern China (Table 2,3), the domestic duck was shown to be the major reservoir of these PMVs (Table 3). The slightly higher the isolation rate from chickens reflects their susceptibility to the virus and the presence of vaccine strains (data not shown). The isolations from ducks were from apparently healthy birds, the isolation rate from the cloaca being about four times that from the trachea (data not shown). Isolations were made all year round but in contrast to influenza, they were generally more frequent in the cooler, winter months. The view has been expressed that the duck which is

Table 3. ISOLATION OF AVIAN PARAMYXOVIRUSES FROM DOMESTIC POULTRY SAMPLED AT A HONG KONG DRESSING PLANT, NOVEMBER 1975 - OCTOBER 1980 (Foo, 1982)

Type of poultry	No. of samples ^a	Isolation frequencies (%)	
		Avian-PMV1 (Newcastle disease Virus)	Other avian PMVs
Duck	8737	2.6	0.5
Goose	1353	1.6	0.3
Chicken	1708	3.1	0.1

^a combined totals of cloacal and tracheal swabs

TABLE 4. ISOLATION OF PARAMYXOVIRUSES FROM REPTILE HOSTS

Host	Symptoms	Reference
Fer-de-lance (<u>Bothrops atrox</u>)	Lethargy, terminal respiratory disease	Foelsch and Leloup (1976) Clark et al. (1979)
Rock rattle snake (<u>Crotalus lepidus</u>)	Progressive central nervous disease, pneumonia	Jacobson et al. (1980)
Family Viperidae members of genera <u>Crotalus</u> , <u>Vipera</u> , <u>Bothrops</u> , <u>Trimeresurus</u> , <u>Bitis</u>	Gaping mouth; convulsions; mucoid exudate throughout respiratory tract	Jacobson et al. (1981)
Viperids and elaphids (cobras)	Not recorded but indicative of paramyxovirus infection	Jacobson et al. (1986)
Ottoman pit vipers (<u>Vipera xanthina xanthina</u>)	Pneumonia, mucous in mouth	Potgieter et al. (1987)

raised in close proximity to domestic poultry in the region is the major source of infection for chickens (Riddell-Swan and Shortridge, 1985).

While other birds may carry avian PMV-1, (Alexander et al., 1984), their role in the spread of disease to domestic chickens is not always that clear because of spread through the agency of man (Lancaster and Alexander, 1975). Details on spread and transmission are considered elsewhere (Alexander, 1982b; Lancaster and Alexander, 1975; Shortridge, 1982c).

As with influenza, the main point to emerge is that the domestic duck of southern China is the major host or reservoir of avian PMVs, especially avian PMV-1.

REPTILIAN HOSTS

Paramyxoviruses have been isolated from viperid snakes with respiratory symptoms mainly from dieoffs in collections (Table 4). There is a noticeable absence of isolations from other snakes held in the vicinity. Jacobson (1986) did, however, report that several elaphids in a major dieoff in a collection of several hundred viperids also died with clinical signs typical of paramyxovirus infection.

Preliminary studies indicate that the virus isolated from Ottoman vipers is antigenically related to parainfluenza 2 virus (Potgieter et al., 1987). These data, albeit somewhat limited, presently indicate that reptile paramyxoviruses may have a restricted host range. On the other hand, serological studies using the haemagglutination inhibition (HI) test, suggest that they may infect a wide variety of species (Jacobson, 1986). However, the interpretation of HI serology in such animals must always be treated with caution because of false positives arising from the possible incomplete removal or inactivation of nonspecific inhibitors of ha-

emagglutination (NSI). Avian sera contain little NSI activity, if any, for avian ortho- and paramyxoviruses (Shortridge, unpublished data) and it remains to be seen if the same applies to reptile sera.

REPTILE-BIRD ANCESTRAL LINKS?

The recognition that waterfowl, notably the domestic duck of southern China, are host to a vast reservoir of influenza and paramyxoviruses begs the question of these virus's ancestral hosts. It is perhaps appropriate to take account of the link between reptiles and birds notwithstanding the limitation of the geological record and its interpretation.

There has been little doubt that birds originated from reptiles. The major area of concern has been which group of archosaurs, namely the crocodiles, thecodonts or theropods, is most closely related to the avian lineage. Renewed interest in this field points strongly to the theropods, i.e. the predaceous dinosaurs, being the most likely ancestor to the class Aves. Osteological chambers that were once regarded as uniquely avian, e.g. furcula, hypotarsus, have now been identified in various theropod dinosaurs and it has been argued by Benton (1985), Thulborn (1984) and Thulborn and Hamley (1982) that some of these animals were actually more bird-like than Archaeopteryx. An outline phylogeny of diapsids is shown in Figure 1.

The early radiations from the thecodonts are far from clear. This is reflected in Olsen's grouping of birds into "basal" and "higher" land bird assemblages, and "water" bird assemblages (Olson, 1985).

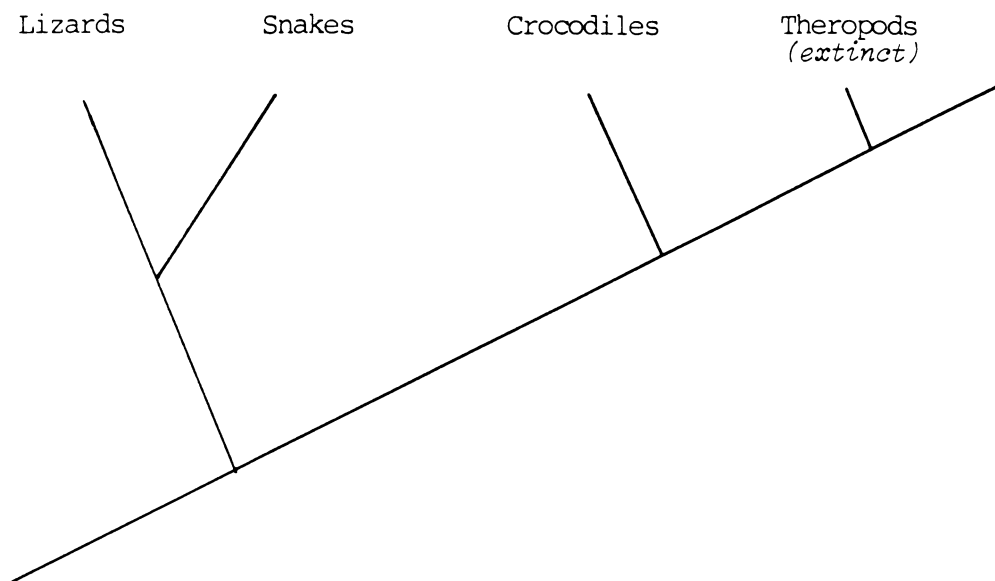


Figure 1. An outline phylogeny of diapsids (adapted from Benton (1986), Thoulborn (1984) and Hamley, 1982)

Figure 2 gives a somewhat simplified phylogeny adapted from this based on splitting points rather than geological time. The earliest basal land birds are considered to have arisen before the other birds and to have continued. Nevertheless, there is evidence that in early avian history there do appear to be aquatic, water-loving birds but whether these represent a primitive stage is open to question (Feduccia, 1980) since the possibility that they are an artefact of the fossil record cannot be excluded (Thulborn, personal communication). They do, however, represent a possible link for the water birds that followed. Thus, it is relevant to note that the most conclusive fossil evidence of an early separation comes from recent work which traces the origin of the Anseriformes (waterfowl) back to a group of highly colonial Charadriiformes (shorebirds) that adapted to shallow saline lakes and evolved a unique filter feeding mechanism (Olson and Feduccia, 1980). Put simply, the waterfowl comprise a very successful group of birds with a long evolutionary history that includes the most vagile birds and the sedentary Muscovy duck and its domestic varieties.

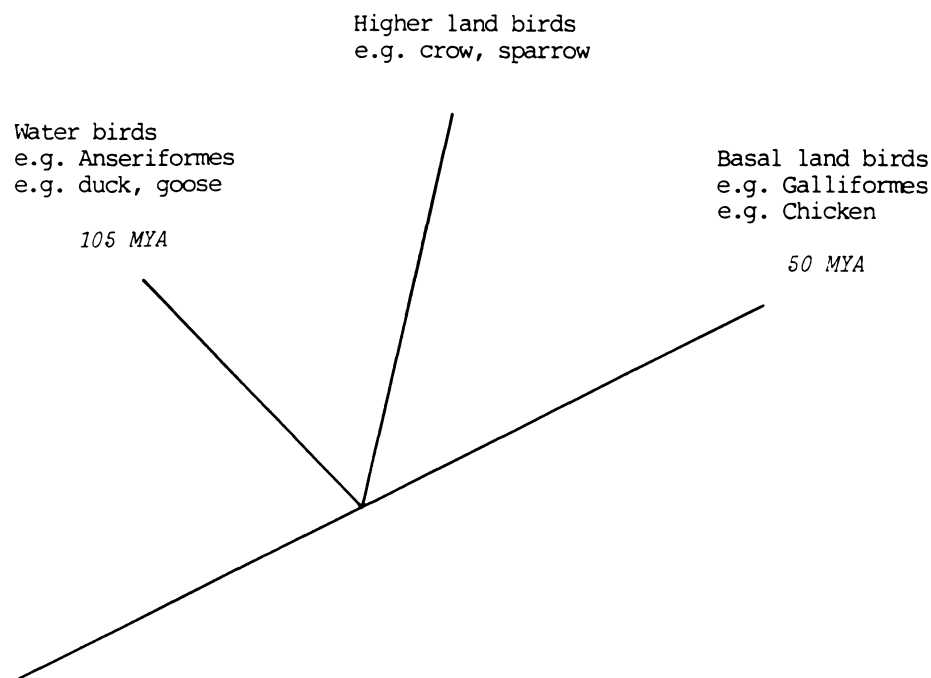


Figure 2. An outline phylogeny of recent birds adapted from Olson (1985) and Molnar and Archer (1984) including representative age estimates by DNA-DNA hybridization (Sibley and Ahlquist, 1986)

(MYA = million years ago)

When the fossil record is viewed against emerging data based on DNA-DNA hybridization (of modern birds), the aquatic domestic duck appears to be much older than, say, land based domestic poultry such as chickens and turkeys. Making interpretations from the data of Sibley and Ahlquist (1986), this difference is at least 55 million years. These data are based on evidence which suggests that the average rate of DNA evolution (base pairs) is the same in all lineages of birds. Sibley and Ahlquist (1986) assert that it takes about 4.5 million years for the genomes of two species to become different at 1% of their base pairs after they have diverged from a common ancestor.

DISCUSSION

Although evidence suggests that there is a reptile-theropod - early land bird (?) - water bird lineage, it does not necessarily follow that each step in the lineage gave rise to the next's pathogens, in this case influenza and paramyxoviruses. The theropods, now extinct, are the missing link in tracing viral ancestors. Any attempt to trace the ancestral origin of these viruses must be speculative; this is compounded by the paucity of isolations from modern reptiles.

While influenza and paramyxoviruses are remarkably successful in their relationship with present day aquatic birds, there is no way of telling whether a similar successful relationship existed with the theropods. Influenza and paramyxoviruses cause short-lived infections and can only be sustained in animals that live in high population density with high turnover such as the domestic duck of southern China (Shortridge, 1982a and 1983).

Whether they could have sustained in theropods in the same way is speculative. However, it is noteworthy that dinosaur remains have very recently been discovered in China which suggest that these animals lived in large herds that migrated across an ancient land mass that joined North America and Asia (Anon, 1988). Of course, these viruses may have undergone evolution but available biological and molecular data does not afford any insight into this.

It is of interest that two taxonomically related human viruses, namely measles and mumps, can cause serious infections. Measles has the ability to spread rapidly especially in non-immune populations causing extensive morbidity and mortality while mumps can give rise to sterility in a small proportion of adolescent males. The possibility that a virus infection could have led to the demise of the dinosaurs is worthy of further thought!

The phylogeny as given in Figure 1 places snakes much further away from birds than crocodiles. This might account in an evolutionary sense for the very limited number of influenza and paramyxovirus isolations from them (Table 4). The fact that these isolations have been from die-offs in collections (see Jacobson, 1986) and that free living snakes generally do not live in communities suggest that the prospect for many isolations is not high. Even in the case of togaviruses and flaviviruses which are transmitted by insects and might therefore be expected to have a higher order of occurrence in reptiles, the isolation rate is low. This is influenced by a number of factors including season and temperature (see Shortridge and Oya, 1984) which might explain the low isolation rate. Attempted isolation from the garter snake Thamnopsis sp. which undergoes a yearly migration in vast numbers in Canada might prove useful. In so far as the crocodiles being a closer living relative is concerned, this would be technically formidable. There are large high-density crocodile farms in some tropical countries and it would be worthwhile finding out if clinical manifestations similar to those described in Table 4 occur in the crocodiles there as well as attempting virus isolation from them.

The study of viruses of reptiles is relatively new; the very low isolation rate may also reflect an inappropriate site of sampling and isolation system. As it is now known that both avian influenza and paramyxoviruses can be isolated with relative ease from the cloaca and faecal matter of ducks, the intestinal lining and contents of snakes should be investigated for virus. The likelihood of virus replicating in the intestine would depend in part on its inherent stability and time for it to reach the lower intestine unless, of course, it might also reach them by venereal route. Avian influenza viruses are more stable than mammalian ones at low pH; while passage into the lower intestine of ducks can occur in a matter of hours (Webster et al., 1978). The successful use of snake embryos (Clark et al., 1979) and reptile cell cultures (Jacobson et al., 1981) at lower temperatures for virus isolation illustrate the need for this aspect to be explored further.

The isolation of influenza-like viruses from ticks (Haig et al., 1965) with segmented negative sense single stranded RNA (Clerx et al., 1983) that can reassort in both vertebrate (Jones et al., 1987) and invertebrate (Davies et al., 1987) hosts adds another dimension to the ecological perspective of influenza (and parainfluenza) viruses. One of these influenza-like viruses, Thogoto virus, causes abortion in sheep but is able to spread rapidly in large flocks suggesting that it was unlikely to have been entirely tick transmitted (Davies et al., 1984).

This raises the question whether invertebrate vectors which may feed on reptiles can transmit influenza and paramyxoviruses in the same way as they do togaviruses and flaviviruses (Shortridge and Oya, 1984). This suggests that the net needs to be cast wider for attempted virus isolation studies. Experimental infection of reptiles and ducks with a range of influenza and paramyxoviruses may provide informative data on ancestral host links.

All in all, there can be little doubt that influenza and paramyxoviruses are remarkably successful parasites of the aquatic duck which is probably an evolutionary repository for them. If their prime natural habitat in the duck is the intestine, then infection of other birds and animals via the respiratory route may give rise to recognizable infection best exemplified by disease outbreaks in domestic turkeys and chickens. It is conceivable that a similar situation also exist in the lower vertebrates. Increased virus surveillance of reptiles via intestinal and respiratory routes will be informative and may help shed light on the ancestral origin of these successful avian viruses.

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