INFECTION AND IMMUNITY, Nov. 1981, p. 354-361
0019-9567/81/110354-08$02.00/0

Vol. 34, No. 2

Replication of Avian Influenza A Viruses in Mammals

VIRGINIA S. HINSHAW,[1]* ROBERT G. WEBSTER,[1] BERNARD C. EASTERDAY,[2] AND WILLIAM J. BEAN, JR.[1]

St. Jude Children's Research Hospital, Memphis, Tennessee 38101,[1] and University of Wisconsin, Madison, Wisconsin 53706[2]

Received 16 March 1981/Accepted 22 June 1981

The recent appearance of an avian influenza A virus in seals suggests that viruses are transmitted from birds to mammals in nature. To examine this possibility, avian viruses of different antigenic subtypes were evaluated for their ability to replicate in three mammals—pigs, ferrets, and cats. In each of these mammals, avian strains replicated to high titers in the respiratory tract (10^5 to 10^7 50% egg infective doses per ml of nasal wash), with peak titers at 2 to 4 days post-inoculation, similar to the pattern of human and other mammalian viruses in these animals. Most avian strains were recovered for 5 to 9 days post-inoculation. One avian H1N1 virus initially replicated poorly in pigs, but was adapted to this host and even transmitted to other pigs. Replication of the avian viruses occurred in the respiratory tracts of mammals, whereas, in birds, they replicate in the intestinal tract as well. The infected mammals had no significant disease signs and produced low levels of humoral antibodies; however, challenge experiments in ferrets indicated that they were immune. These studies suggest that influenza A viruses currently circulating in avian species represent a source of viruses capable of infecting mammals, thereby contributing to the influenza A antigenic pool from which new pandemic strains may originate.

Avian influenza A viruses have not been shown convincingly to replicate or produce disease in mammals, yet the recent isolation of an "avian" virus from sick harbor seals (*Phoca vitulina*) suggests that this can occur in nature (9a; J. R. Geraci, D. J. St. Aubin, R. G. Webster, V. S. Hinshaw, W. J. Bean, H. L. Ruhnke, S. Madoff, I. K. Barker, and J. R. Prescott, submitted for publication; R. G. Webster, V. S. Hinshaw, W. J. Bean, K. L. van Wyke, J. R. Geraci, and G. Petursson, Virology, in press). The seal virus, which is antigenically related to fowl plague virus, possesses all eight gene segments most closely related to avian viruses (Webster et al., in press). In view of this event, it is clear that studies are needed to examine the potential of avian viruses to replicate in mammals.

Extensive studies (4, 19) have shown that birds harbor a large number of different influenza A viruses, including strains antigenically (23) and genetically (16) related to those in mammals. Such findings have led to the suggestion that avian viruses may contribute some genes to the evolution of new viruses appearing in mammals, including humans (23), yet there are little data indicating that avian viruses can infect mammals under natural or laboratory conditions. To analyze this possibility, we have examined (i) the ability of avian influenza A viruses of different antigenic subtypes to replicate in pigs, cats, and ferrets, (ii) the adaptation and transmission of an avian virus in pigs, and (iii) antigenic and genetic comparisons of viruses after passage in different hosts. The results indicate that most of the avian viruses tested can replicate in mammals, suggesting that interspecies transmission of these viruses from birds to mammals could occur in nature.

MATERIALS AND METHODS

Viruses. The antigenic classification of the viruses used in this study follows the recent World Health Organization recommendations (27); the previous classification (25) is also included. The influenza A virus strains used in this study were from the repository at St. Jude Children's Research Hospital and are identified under appropriate sections below.

Serological tests and virus identification. The influenza viruses were identified in hemagglutination inhibition (HI) tests (12) and neuraminidase inhibition (NI) tests (26) with specific antisera to the isolated surface antigens of reference influenza viruses. Antisera to selected avian isolates were prepared in ferrets by standard procedures (12). When the HI titers of the ferret sera were <1:20 at 12 days post-inoculation (p.i.), the ferrets received an intraperitoneal injection of 5×10^7 50% egg infective doses (EID_{50}) of the same virus and were bled 10 days later. It should be noted

that the intraperitoneal boost was necessary to produce a significant antibody response (HI titer of >1:80) in ferrets inoculated with avian strains, and even this approach has not always been successful.

In vivo virus replication studies. Ferrets, cats, pigs, turkeys, and mallard ducks (all from 1 to 4 months of age) had no serological evidence of prior exposure to influenza viruses, and no viruses were isolated from samples taken before inoculation. The mammals were inoculated intranasally, and the birds were inoculated orally and intratracheally with 10^6 to 10^8 EID_{50} of virus. The animals were examined daily for clinical signs of disease. Cloacal, tracheal, rectal, or nasal swabs were taken daily from inoculated animals, treated with antibiotics, and assayed for influenza viruses as previously described (5). Nasal washes from the mammals were collected as described (14) except that, in these studies, the animals were anesthetized with Ketamine (Bristol Laboratories) before sampling to facilitate handling and increase mucous secretions. Nasal washes were titrated in embryonated chicken eggs to determine virus concentrations (EID_{50} per milliliter). Organs were collected aseptically for virus titrations. The tissues were weighed, ground in a mortar with powdered glass, diluted, and titrated for infectious virus in embryonated chicken eggs. Sera from the mammals at 14 days p.i. were examined for antibodies in HI and NI tests.

RESULTS

Replication of A/Dk/Alb/573/78 (H1N1) in mammals and birds. Previous studies in our laboratory (7, 22) and others (11) suggest that avian viruses that are antigenically related to swine strains replicate in pigs. In these studies, however, replication was based solely on virus recovery, i.e., the presence or absence of a virus in nasal swabs, which provided no quantitative evidence of virus replication. Pigs and ferrets were inoculated with an avian H1N1 (Hsw1N1) virus, A/Dk/Alb/573/78 (Dk/573), and virus replication was monitored by titrating the infectious virus in individual nasal washes collected daily from these animals. Since ferrets are very susceptible hosts to human influenza viruses (14), it was of interest to examine their susceptibility to an avian strain. The results (Fig. 1) showed that, at 2 h p.i., there were low levels of virus in the nasal washes of the animals, but the titers increased markedly between 1 to 3 days p.i. In ferrets, the levels of infectious viruses increased during the first 3 days, with peak titers (10^7 EID_{50}) at day 3 p.i., and then declined until no virus could be recovered by day 9 p.i. Dk/573 reached higher titers and persisted in the ferrets longer than the human strain, A/Hong Kong/1/68 (H3N2) (HK/68) (Fig. 2). In pigs, the peak virus titer ($10^{5.5}$ EID_{50}/ml) occurred on day 1 p.i. in one and day 3 p.i. in the other (Fig. 1). Although avian viruses replicate in the intestinal tracts of birds (24), no viruses were detected in

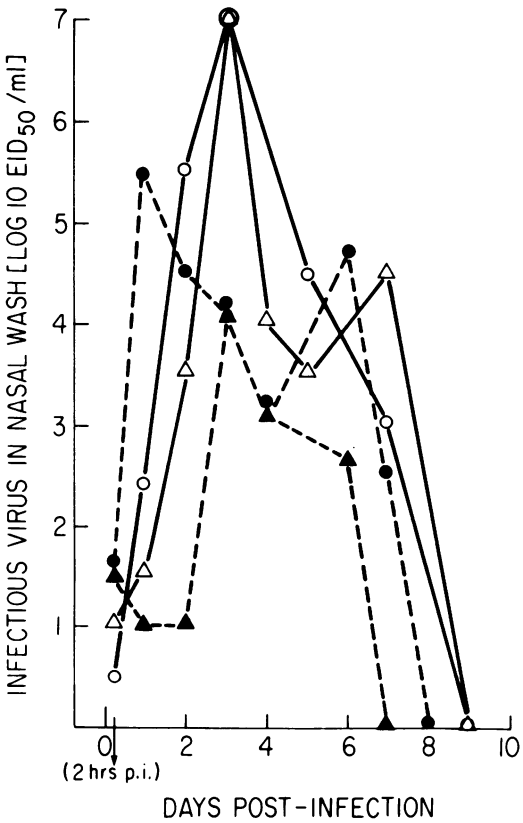

FIG. 1. *Replication of Dk/573 in ferrets and pigs. Two ferrets were inoculated intranasally with 10^7 EID_{50}, and two pigs were inoculated with 10^8 EID_{50} of Dk/573. Nasal washes were collected daily and titrated in embryonated chicken eggs. Symbols: △, ○, Results from individual ferrets; ▲, ●, results from individual pigs.*

rectal samples from the pigs or ferrets. Pigs showed no disease signs, whereas ferrets experienced increased nasal discharges from 2 to 4 days p.i.

To determine whether Dk/573 replicated in the lower respiratory tract, ferrets were sacrificed on days 3, 5, and 7 p.i., and nasal turbinates and trachea and lung tissues were collected for virus titration. The results (Table 1) showed that the lungs and tracheae contained high titers of viruses ($10^{6.2}$ EID_{50}/g) on day 3 p.i.; the virus titers in these tissues were greatly reduced but not totally eliminated by day 7 p.i. The nasal turbinates also yielded viruses through day 7 p.i. No viruses were recovered from blood or rectal samples. These results indicate that Dk/573 replicated in both the upper and lower respiratory tracts of the ferrets.

Since Dk/573 replicated so efficiently in pigs and ferrets, it was necessary to confirm that Dk/

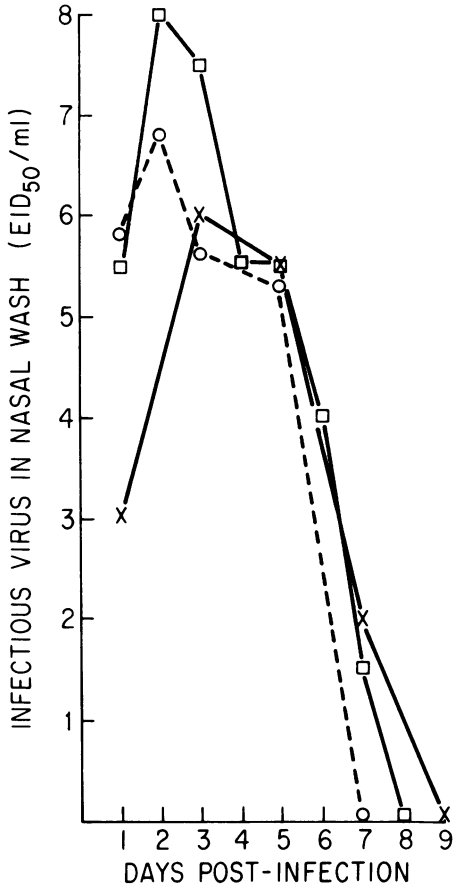

FIG. 2. *Replication of avian and mammalian influenza viruses in ferrets. Ferrets were inoculated intranasally with 10^6 EID_{50} of Dk/Iceland, Seal/Mass, or HK/68. Nasal washes were collected and titrated in embryonated chicken eggs. Symbols: ×, Dk/Iceland; □, Seal/Mass, ○, HK/68.*

573 was an avian virus. Since avian, but not mammalian, influenza viruses replicate in the intestinal tracts of birds (24), turkeys and ducks were inoculated orally and intratracheally with Dk/573, and tracheal and cloacal samples were collected for virus isolation. Viruses were recovered from both the tracheae and cloacae of the ducks and turkeys for 8 days p.i. (data not shown). These results demonstrated that Dk/573 possessed the tissue tropism of avian influenza virus in birds. On the other hand, since Dk/573 was not recovered from rectal samples from mammals, it was clear that this virus did not replicate in the intestinal tracts of these mammals.

Comparison of the replication of avian, swine, and human isolates in pigs. Since the above studies clearly demonstrated that the avian virus Dk/573 replicated in pigs, we next examined whether other avian H1N1 viruses, isolated at different times and geographical locations, could also infect pigs. In this experiment, pigs were inoculated intranasally with six avian strains and, for comparison, two related viruses isolated from pigs and humans, A/Sw/Wis/1/67 and A/NJ/8/76. Nasal and rectal swabs were collected for virus isolation for 10 days. The results (Table 2) showed that four of the avian viruses and the human and swine viruses were recovered for 6 to 7 days p.i. A cloned preparation of A/Dk/Alb/35/76 (Dk/35) was recovered for only 1 day p.i., even less than the 3 days p.i. for the uncloned Dk/35. No viruses were isolated from the rectal samples from the pigs. The pigs infected with human and swine isolates had fevers (>40°C) but no other significant disease signs, whereas the pigs infected with the avian strains displayed no disease signs.

TABLE 1. *Recovery of Dk/573 (H1N1) from the respiratory tracts of ferrets*[a]

Day p.i.	Virus titer (log_{10} EID_{50}/g) in following tissue:		Virus recovery[b] from following sample:			
	Lung	Trachea	Rectal swab	Blood	Nasal swab	Nasal turbinates[c]
3	6.2	5.7	−	−	+	+ (2.2)
5	3.6	2.0	−	−	+	+ (2.7)
7	2.1	1.6	−	−	−	+ (1.0)

[a] Ferrets were inoculated intranasally with approximately 10^7 EID_{50} of Dk/573, and swabs and tissues were collected and assayed as described in the text.

[b] +, Virus was recovered; −, no virus was detected.

[c] Nasal turbinates were scraped into 3.0 ml of phosphate-buffered saline. The virus titers (log_{10} EID_{50} per milliliter) of these samples are shown within parentheses.

TABLE 2. *Recovery of avian H1N1 (Hsw1N1) viruses from experimentally inoculated pigs*[a]

Inoculum	Virus recovered from nasal passages (days p.i.)
Dk/Alb/35/76	1-3
Dk/Alb/35/76 Clone I[b]	1
Dk/Alb/46/77	1-6
Dk/NJ/1853/78	1-6
Dk/573	1-7
Dk/Alb/714/78	1-7
Sw/Wis/1/67	1-7
NJ/8/76	1-7

[a] Two pigs were inoculated intranasally with approximately 10^8 EID_{50} of virus (see the text). Nasal swabs were collected for 10 days p.i. and inoculated into embryonated chicken eggs for virus isolation.

[b] This virus stock was obtained by three limit dilution passages of Dk/35 in embryonated chicken eggs.

These findings indicated that avian strains that were antigenically related to swine virus replicated in pigs without producing disease. Although quantitative studies were not done on each of these viruses, the length of virus shedding, detected by nasal swabs and nasal washes, agreed closely (e.g., Dk/573), indicating that these avian viruses were replicating. Also, the avian strains were recovered for as long as the human and swine viruses.

Adaptation of Dk/35 to pigs and subsequent transmission to contact pigs. The above studies (Table 2) suggested that, in contrast to the other avian strains, Dk/35 clone I replicated poorly, if at all, in pigs. To determine whether in vivo passage of this virus would result in the selection of a strain that would replicate, the virus was sequentially passed six times in pigs. For these studies, Dk/35 clone I was used as the initial inoculum, and then a virus recovered in eggs from nasal swabs was used to inoculate the next pig. During passage, viruses were recovered for increasingly longer periods, up to 5 days p.i. Pig 6 was sacrificed at day 3 p.i., and a virus was recovered from the lungs of this animal. These results indicated that in vivo passage increased the recovery time, suggesting that even this avian strain, which initially replicated poorly, could be adapted to pigs.

To determine whether this "pig-adapted" avian virus could be transmitted to other pigs, 12 pigs were inoculated with the virus from passage 6, and, after 24 h, the animals were put in contact with 12 uninoculated pigs. Nasal and rectal samples were taken daily. On days 3 to 8 p.i., inoculated and contact pigs were sacrificed at random and autopsied for evidence of gross pathological lesions. Viruses were recovered from the nasal passages of six inoculated pigs for as long as 5 days p.i. and from two of the contact pigs during the 8 days of exposure. No viruses were detected in rectal samples. Autopsies revealed no significant gross pathology in the lungs of the animals, although one infected pig had small lesions at day 5 p.i. Pigs experimentally infected with swine viruses (A/Sw/Wis/1/67 and A/NJ/8/76) showed no significant lung pathology. These findings indicated that pigs infected with the avian virus could transmit the virus to other pigs.

To determine whether antigenic differences between the original Dk/35 clone I and the pig-adapted virus could be detected, these viruses were compared in HI and NI tests with rabbit, chicken, and ferret antisera. Even with the ferret antisera, the HI and NI reactions of Dk/35 clone I and the pig-adapted virus were indistinguishable (data not shown). The ribonucleic acids of these viruses were compared by polyacrylamide gel electrophoresis as previously described (18), and the ribonucleic acid migration patterns of the viruses were indistinguishable. By these assays, antigenic or genetic changes in the virus which may have occurred during passage were not detected. More sensitive techniques, such as oligonucleotide mapping, are currently being used to examine these viruses.

Replication of avian and mammalian influenza viruses of various antigenic subtypes in mammals. Although the above studies might suggest some correlation between the antigenic relatedness of the avian and swine viruses with the ability to replicate in pigs, there is no a priori reason that this should be so. Other avian strains of various antigenic subtypes were, therefore, examined in three mammalian species. In these studies, the replication of avian viruses was compared with those of the human virus HK/68 and the seal isolate A/Seal/Mass/1/80 (H7N7) (Seal/Mass). The seal virus was of particular interest since this virus, although genetically most closely related to avian viruses, was associated with a disease outbreak in marine mammals (Geraci et al., submitted for publication). It is likely that this virus spread from birds to seals in nature, emphasizing the possible significance of avian viruses for mammals. Also, the seal virus could possibly spread to other mammals, so it was important to evaluate this potential.

(i) Ferrets and pigs. Since ferrets and pigs were susceptible to the avian H1N1 viruses, it seemed reasonable to include them in these studies. Also, pigs are economically important domestic animals, so the possibility that avian or seal viruses could infect and even cause disease in them needed to be evaluated. Ferrets were inoculated with HK/68, Seal/Mass, and Dk/Iceland/29/80 (H7N7) (Dk/Iceland) viruses, and nasal washes were collected and titrated for infectious virus. In ferrets (Fig. 2), the HK/68 and Seal/Mass viruses both reached peak titers within 2 days p.i. ($10^{6.8}$ and $10^{8.0}$ EID_{50}/ml, respectively), whereas the Dk/Iceland virus was recovered at slightly lower titers, with the peak at day 3 p.i. ($10^{6.0}$ EID_{50}/ml). HK/68 could no longer be recovered by 7 days p.i., whereas the avian and seal viruses were still present at low levels, but could not be recovered after day 7.

Since Dk/Iceland replicated in ferrets, a number of other avian strains were also examined. In this case, virus shedding was detected by virus isolation from daily nasal swabs. The results (Table 3) showed that the avian strains were recovered from 3 to 7 days p.i., suggesting

TABLE 3. *Virus recovery and antibody responses of mammals inoculated with avian and mammalian influenza A viruses*[a]

Inoculum	Virus recovered from nasal passages (days p.i.)		HI antibody response[b] of ferrets
	Ferrets	Pigs	
Avian			
Dk/Ont/77 (H2N1)	1-3	ND[c]	80
Dk/Alb/133/78 (H6N2)	1-3	ND	20
Dk/NY/6750/78 (H2N2)	1-5	ND	20
Dk/Alb/604/78 (H2N3)	1-5	1-5	<20
Dk/NY/6874/78 (H3N2)	1-5	1-5	<20
Tk trough/Minn/3/79 (H10N7)	1-5	ND	20
Gull/Mass/1/80 (H?N6)	1-5	ND	20
Dk/Alb/358/79 (H3N6)	1-6	ND	20
Dk/Iceland/29/80 (H7N7)	1-7	ND	20
Seal			
Seal/Mass/1/80 (H7N7)	1-7	1-7	20
Human			
HK/1/68 (H3N2)	1-5	ND	1,280

[a] Ferrets were inoculated intranasally with 10^7 EID_{50} of virus, and pigs were inoculated with 10^8 EID_{50}. Nasal swabs were collected for 10 days p.i. and inoculated into embryonated chicken eggs for virus isolation.

[b] Ferrets were bled at 14 days p.i. The HI titer is the reciprocal of the highest dilution of sera inhibiting four hemagglutinating doses of virus.

[c] ND, Not determined.

that these viruses, like the H1N1 strains, differ in their ability to replicate. Viruses recovered for as long as 5 to 7 days p.i. included isolates from ducks, turkeys, and gulls, indicating that various avian species harbor viruses capable of infecting ferrets. Although several of the avian strains possessed one or both surface antigens related to human viruses such as H2, H3, N1, and N2, there was no apparent correlation between the length of virus recovery and this antigenic relationship. For example, the duck virus of the H2N1 subtype was recovered for the shortest time (3 days), whereas the turkey and gull viruses, which were unrelated to human viruses, were recovered for as long as the human virus HK/68. Replication of several strains shown in Table 3 have been quantitated by titrating nasal washes from these animals; for example, Dk/Alb/358/79 reached high titers (10^6 EID_{50}/ml of nasal wash) at 3 days p.i., similar to the results with Dk/Iceland. The ferrets infected with these viruses showed no significant disease signs. Since most of the avian strains tested were recovered from ferrets for as long as the human and seal viruses, these studies would indicate that ferrets are susceptible hosts for a wide range of different avian influenza viruses.

Pigs were inoculated with two avian viruses and Seal/Mass (Table 3). Both avian viruses were recovered for 5 days p.i., similar to the avian H1N1 viruses. Seal/Mass was recovered for even longer, i.e., 7 days p.i., and high titers (>$10^{6.5}$ EID_{50}/ml) of virus were detected in nasal washes from days 2 to 4 p.i. The pigs infected with these viruses showed no disease signs. These results indicated that pigs support the replication of several different influenza A viruses from avian and mammalian species.

(ii) Cats. In view of the replication of the avian viruses in both pigs and ferrets, the question arose as to whether other mammals, particularly those in close contact with humans, were susceptible to avian strains. Preliminary studies by Paniker et al. (13) indicate that cats are susceptible to infection with human H3N2 viruses. Cats were, therefore, inoculated with three viruses isolated from different species, Ty/Ore/1/71 (H7N3) (Ty/Ore), Seal/Mass, and HK/68. Nasal washes were collected and titrated for virus. The results (Fig. 3) showed that Seal/Mass replicated to high titers ($10^{6.5}$ EID_{50}/ml) by 3 days p.i., whereas Ty/Ore and HK/68 did not reach peak titers ($10^{5.5}$ EID_{50}/ml) until 5 and 6 days p.i. All of these viruses were recovered for 8 and 9 days p.i. The cats showed no disease signs. These findings indicated that these influenza viruses from birds, seals, and humans all replicated in cats.

Serological responses of mammals infected with avian viruses. Although the above studies showed that avian and also seal viruses replicated as well as human viruses in the mammals examined, there were differences in the immune responses of these animals. Ferrets receiving the human virus HK/68 produced high levels of antibodies (HI titer of 1:1,280), whereas ferrets infected with avian viruses produced little, if any, detectable antibody (HI titers of <1:20 to 1:80) (Table 3). Even Seal/Mass, which reached high titers and persisted longer in the ferrets than did HK/68 (Fig. 2), did not induce a significant antibody response (HI titer of 1:20). Similar results were obtained with sera from pigs and cats (data not shown). On the other hand, it was found that ferret sera had significant levels of antibody to neuraminidase. Ferrets infected with Dk/573 had serum HI titers of 1:20 but NI titers of 1:100. Whether these results reflected a reduced immunogenicity of avian hemagglutinin molecules or low antibody avidity is not known.

Since the antibody responses of the animals

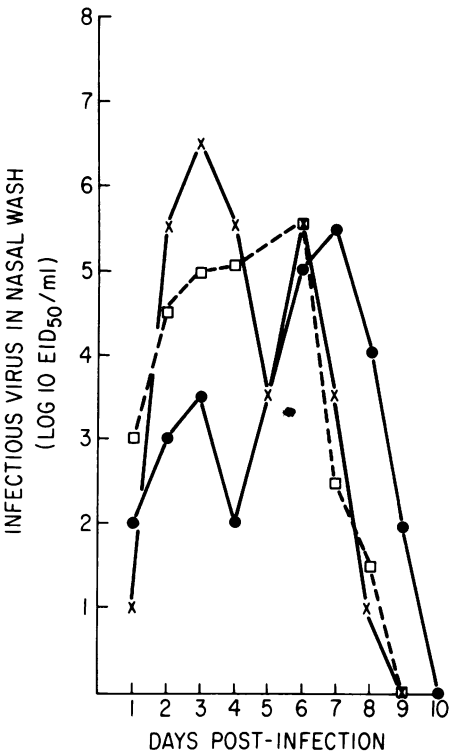

FIG. 3. *Replication of avian and mammalian influenza viruses in cats. Cats were inoculated intranasally with 10^7 EID_{50} of Ty/Ore, Seal/Mass, and HK/68. Nasal washes were collected and titrated in embryonated chicken eggs. Symbols: ×, Seal/Mass; ●, Ty/Ore; □, HK/68.*

were low, it was possible that they were still susceptible to infection. To examine this, ferrets were reinfected with Dk/573 2 weeks after the initial infection (Fig. 1). No viruses were recovered from nasal swabs during the next 4 days, indicating that these animals were immune, although the levels of humoral antibodies were low.

DISCUSSION

The above studies showed that currently circulating avian influenza A viruses of various antigenic subtypes replicated in pigs, ferrets, and cats to the same level as human influenza viruses. These findings demonstrated that avian viruses can infect mammals, supporting the possibility that this could occur in nature.

Previously, there had been little evidence that avian influenza viruses could infect mammals. Early laboratory studies (20) suggested that two virulent avian viruses, A/tern/S.A/61 and A/turkey/England/63, produced disease in mice. Only one human infection with an avian virus has been reported (3); in this case, fowl plague virus was isolated from the individual, but no serological evidence of infection was detected. More recently, studies (7, 11, 22) have shown that duck isolates, antigenically related to swine viruses, could be recovered for 5 to 8 days p.i. from experimentally infected pigs. Our studies provided quantitative evidence that such avian strains replicated to high titers in pigs, similar to swine viruses. Also, one avian virus was transmitted to other pigs, suggesting that an avian virus can survive in a pig population. One may postulate that the current swine viruses originated from avian strains; however, this may have occurred long ago, since pigs obviously maintain these viruses (4, 5). Whether the transmission of viruses from birds to pigs occurs in nature remains unknown.

Ferrets were susceptible to a wide range of different avian viruses, including isolates of various antigenic subtypes from different avian species. Many of these viruses replicated to higher titers and persisted longer than the human virus HK/68; however, no significant disease signs or antibody responses were produced in either mammals or birds infected with these strains. In contrast, ferrets infected with HK/68 displayed obvious disease symptoms and produced high levels of humoral antibodies. The reasons for these differences are not understood. It could be postulated that the replication of avian viruses produces minimal cellular destruction, resulting in a weak antigenic stimulus; however, this would not explain the finding that the ferrets were immune. It is possible that the other arm of the immune response, i.e., cell-mediated immunity, may be more important than humoral antibody in these infections in both birds and mammals. This aspect has not yet been evaluated.

Our studies on cats confirmed an earlier report (13) that human H3N2 viruses replicate in these animals. In addition, cats were susceptible to an avian virus and another mammalian virus (Seal/Mass). These findings suggest that cats represent yet another host for various influenza A viruses in nature. Since cats have frequent contact with humans, as household pets, and with birds, as predators, it is possible that cats could be a vehicle for transmission of viruses between avian and mammalian species, but this has not yet been examined.

The ability of the avian viruses to replicate in pigs, ferrets, and cats underlines the possibility that the avian reservoir serves as a source of viruses appearing in mammals. The recent detection of an avian virus in seals (9a; Webster et al., in press) represents a likely example of such

a transmission in nature. This raises the question as to the potential of avian viruses and the seal virus with avian genes to transmit to humans. During experimental and field studies with infected seals, several investigators experienced conjunctivitis, and high titers of the seal virus were recovered from the eye of one individual (21), indicating that the seal virus can infect human eyes. With regard to the avian isolates, human volunteer studies by Beare (personal communication) suggested that most avian strains did not infect individuals inoculated intranasally; however, two viruses, including Dk/573 used in our studies, were shed by the volunteers, suggesting that limited replication may have occurred. Although these results would suggest that transmission of avian viruses to humans seems unlikely, it would be premature to eliminate such a possibility, since the requirements for virus replication and survival in a population are not clearly defined at this time.

Our studies on the ability of these avian viruses to infect mammals relate to current efforts to understand the viral and host factors which dictate the host range of a virus. Studies have shown that such factors as cleavage of the hemagglutinin (9, 10) and a compatible combination of hemagglutinin and neuraminidase (2, 17) are important for optimal infectivity of these viruses; and that a single polymerase gene (P3) determines the host range of a virus in tissue culture (1); however, these would not appear to be the only important features. Studies by Scholtissek et al. (15) have shown that the host range of a virus may be changed and then regained by genetic reassortment between strains, although the virus retains the same hemagglutinin. This may be quite significant in nature, since it would increase the chance for virus survival. It may be particularly relevant in the avian species, in which a large pool of influenza viruses continually circulates (8) and genetic reassortment between viruses occurs quite readily (6), thereby producing a genetically diverse population of viruses (18). Possibly, this situation contributes to the perpetuation of these viruses in the many different species of birds in the world and extends the host range of some strains to mammals. Additional studies are required to elucidate the genetic basis of such a complex biological property as host range.

These studies indicate that the host range of avian influenza A viruses is broad and includes both birds and mammals. In view of this, the distinction between avian and mammalian influenza A viruses might be artificial. The possibility that the host range of avian viruses includes different mammals in nature gains support from the appearance of an avian virus in seals. The potential exists that other mammals, including humans, may be involved in the circulation of these avian viruses in nature.

ACKNOWLEDGMENTS

We wish to acknowledge the excellent technical assistance of Jim Bigelow and Carol Anne Bockhold.

This work was supported by contract AI 02649 from the National Institute of Allergy and Infectious Diseases, by Cancer Center Support (CORE) grant CA 21765 from the National Cancer Institute, and by ALSAC.

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