tainties, the present work indicates that net influx of amino acids from dilute solution in seawater has the potential to contribute substantially to the carbon and nitrogen requirements of the larvae of *S. purpuratus*.

Since there were no bacteria present in the experimental material used in our study, the larvae must be the biological agent responsible for the observed removal of substrate. Influx of ^{14}C-labeled leucine and serine occurs at rates identical with those observed for net removal of these substrates. Thus, our data provides support for previous observations on uptake and assimilation of DOM by nonaxenic marine invertebrates and for observations based on influx of labeled substrate (*7*).

DONAL T. MANAHAN
JAMES P. DAVIS
GROVER C. STEPHENS
Department of Developmental and Cell Biology, University of California, Irvine 92717

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Swine Influenza–Like Viruses in Turkeys: Potential Source of Virus for Humans?

Abstract. *Influenza A viruses (subtype H1N1), recently isolated from turkeys in different areas of the United States, were determined to be closely related to strains typically associated with pigs. This conclusion was based on comparisons of H1N1 isolates from pigs, humans, ducks, and turkeys with polyclonal and monoclonal antibodies, RNA-RNA competitive hybridization, and replication studies. One of the H1N1 isolates from turkeys caused influenza in a laboratory technician, who displayed fever, respiratory illness, virus shedding, and seroconversion. These results suggest that turkeys as well as pigs are involved in the maintenance of these viruses and their transmission to humans.*

Swine influenza–like viruses (subtype H1N1) are periodically isolated from humans (*1*). There is no doubt that in some cases the viruses in humans originate from pigs. For example, viruses isolated from sick pigs and humans on farms in Wisconsin were antigenically and genetically indistinguishable (*1*). However, there is not always a clear connection between human infections and contact with pigs. The outbreak of swine influenza in soldiers at Fort Dix, New Jersey, in 1976 was not preceded by such contact (*1*), nor was the influenza in a leukemia patient who died of viral pneumonia in Nevada (*2*). This raises the question of whether other sources of swine influenza viruses exist. In this report we describe influenza A isolates (subtype H1N1) from turkeys that are virtually indistinguishable from viruses typically associated with pigs. The potential importance of these turkey viruses in the epidemiology of human influenza was established by the fact that one of the isolates caused an influenza infection in a laboratory technician.

Two viruses were isolated from six adult female turkeys that showed a sudden drop in egg production in Missouri in 1981. In a separate laboratory other H1N1 viruses were recovered from turkeys that had similar problems with egg production in Missouri, Colorado, and Kansas in 1980 to 1981 (*3*). The viruses were grown in 10- to 11-day-old chick embryos and characterized serologically as H1N1 with hyperimmune goat and rabbit antiserum (*4*).

To determine the antigenic relatedness of these turkey viruses to those in other species (*1–3*), we compared a panel of H1N1 viruses from pigs, turkeys, ducks, and humans in hemagglutination inhibition (HI) tests with antisera from ferrets recovering from infection and with monoclonal antibodies (*5*, *6*). Representative strains of the human, swine, and turkey viruses all reacted at high titers with ferret antisera to the human virus (A/NJ/8/76) and the turkey virus (A/Ty/Mo/1/81) (Table 1). The duck viruses did not react with those antisera. The three monoclonal antibodies that recognize different antigenic determinants on H1 strains show that these determinants are shared by the recent turkey, human, and swine viruses but not by the duck viruses.

To examine the other surface antigen of the turkey isolates (the neuraminidase), we compared the H1N1 viruses in neuraminidase inhibition assays (*7*) with the same ferret antisera. The turkey iso-

lates had a neuraminidase like that of the human and swine viruses. Thus, the serological comparisons of these H1N1 viruses indicate that the turkey viruses differ from H1N1 strains previously isolated from birds and more closely resemble recent swine isolates, such as A/Sw/Wis/8/80, and the related human isolates A/NJ/8/76 and A/Nev/101/82.

To estimate the degree of RNA homology among the turkey, human, and swine strains, we performed competitive RNA-RNA hybridization assays using isolated genome RNA segments from A/NJ/8/76 (Fig. 1). In this assay relative homologies are determined by the efficiency with which various viral RNA's compete with the annealing of a labeled RNA and homologous complementary RNA (*8*). With all genome RNA segments, viral RNA from recent swine isolates and the swine influenza–like turkey and human isolates competed efficiently with the labeled A/NJ/8/76 RNA probes, while other human and avian strains did not. Thus the turkey and human isolates are genetically very similar to recent swine influenza strains.

Since the turkey H1N1 viruses appeared more "mammalian" than "avian," it was critical to study their replication in turkeys. Typically, avian viruses replicate efficiently in the intestinal tracts of birds after oral inoculation and are subsequently shed in high titers in the feces, whereas mammalian viruses do not infect the intestinal tracts of birds (*9*). To assay this biological characteristic, we inoculated turkeys orally and intratracheally with 10^7 median egg-infective doses (EID_{50}) of Ty/Ks/4880/80. We collected and titrated the virus in tissues from the respiratory (nares, trachea, and lung) and intestinal (rectum, cloaca, and feces) tracts of inoculated turkeys 3 days after infection—typically the day of peak virus titers in infected birds (*9*). The only tissue containing a significant titer (3×10^5 EID_{50}) of virus was the nasal cavity—apparently the primary site of virus replication. The turkeys developed significant levels of antibodies (HI titer, 1:40 to 1:160) to Ty/Ks/4880/80 by day 14, indicating infection. Studies of the replication of other H1N1 strains in turkeys showed that (i) the avian H1N1 strain, Dk/Alb/35/76, grew to high titers in both the nasal cavity and the intestinal tract of turkeys and (ii) recent isolates from humans (Nev/101/82) and swine (Sw/Wis/8/80) reached high titers in the nasal cavity—like Ty/Ks—whereas other strains (NJ/8/76 and Sw/Tn/1/75) replicated poorly, if at all. Thus the H1N1 turkey viruses infect and replicate in turkeys but possess a tissue tropism similar to that of mammalian, rather than avian, viruses of the same subtype.

Table 1. Reactions of H1N1 influenza viruses in HI assays with antisera from ferrets recovering from infection (*5*) and with monoclonal antibodies (*6*). Sera and antibodies were treated with receptor-destroying enzyme before their use in the assays. Each value is the reciprocal of the serum dilution inhibiting four agglutinating doses of virus.

Virus	Ferret antiserum to			Monoclonal antibodies to the hemagglutinin of A/NJ/11/76 (χ-53a)		
	NJ/8/76 (post-infection)	Dk/Alb/35/76 (hyper-immune)	Ty/Mo/1/81 (post-infection)	6/1	36/3	30/2
Human						
NJ/8/76	640	80	320	51,200	51,200	3,200
Nev/101/82	640	160	320	12,800	25,600	800
Mem/4/82	1,280	320	640	51,200	51,200	1,600
Swine						
Sw/Ia/15/30	40	20	80	51,200	25,600	< 100
Sw/Wis/8/80	320	40	160	6,400	12,800	400
Avian						
Ty/Ks/4880/80	1,280	320	640	51,200	51,200	1,600
Ty/Mo/1/81	320	160	320	102,400	51,200	3,200
Dk/Alb/35/76	< 40	320	< 40	< 100	< 100	< 100
Dk/Alb/82/81	< 40	320	< 40	< 100	< 100	< 100

During our studies of the H1N1 viruses, a laboratory technician contracted a respiratory illness characterized by fever, myalgia, and nasal congestion. The illness lasted for 5 days; recovery was uneventful and complete. During the acute phase, virus was recovered from a throat swab of this individual; the isolate, A/Mem/4/82, was identified as H1N1 and was antigenically and genetically indistinguishable from Ty/Ks/4880/80 (Fig. 1). In addition, the RNA's of H1N1 strains being studied in the laboratory at that time were compared by polyacrylamide gel electrophoresis (*8*). Comparison of the RNA migration patterns of human, swine, and turkey viruses revealed that only two viruses, A/Mem/4/82 and Ty/Ks/4880/80, had identical patterns. Four weeks after infection the technician had high levels of antibody to Ty/Ks/4880/80 (HI titer, 1:160). Serological monitoring of other laboratory personnel and individuals in contact with the technician produced no evidence of subsequent transmission of the virus.

We conclude that the isolates from turkeys are H1N1 strains similar to cur-

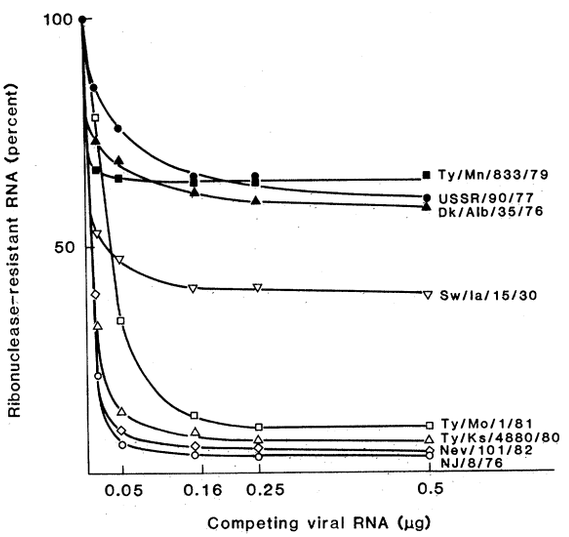

Fig. 1. Comparison of RNA's from avian and mammalian influenza strains with RNA segment 3 from A/NJ/8/76 by competitive hybridization. Iodine 125–labeled RNA segment 3 was annealed with homologous complementary RNA in the presence of increasing amounts of competing RNA from the homologous virus and other strains under study. All viruses were of the H1N1 subtype except Ty/Mn/833/79 (H4N2). The degree of relatedness between the labeled probe and the corresponding RNA segment of the other virus strains is indicated by the efficiency with which each RNA competes with the annealing of the labeled RNA and its homologous complementary RNA. The isolation and iodination of the RNA segments were as described by Bean and colleagues (*8*). The RNA mixtures were annealed for 24 hours at 10°C below the melting temperature of the double strand formed by annealing the labeled probe with homologous complementary RNA. Other details were as described in (*8*). Recent H1N1 swine virus isolates (A/Sw/Tn/1/75 and A/Sw/Minn/1/75) also competed efficiently with the annealing of the NJ RNA. Analysis of the other RNA segments from A/NJ/8/76 gave similar results.

rent isolates from swine. This raises the question as to how these viruses were introduced into the birds. In 1975, when serologic evidence of swine viruses in turkeys was first obtained (*10*), sick pigs on the same farm were suspected as the source. Since swine viruses can infect turkeys, it is feasible that such transmission occurred. More recently, an outbreak of influenza in turkeys in Colorado involved confined birds with no known contact with pigs. It was suggested that humans were the source, having acquired the viruses while slaughtering pigs (*11*). Since swine viruses infect humans (*1*), it is possible that infected farm personnel did transmit the viruses to the turkeys.

The reverse is also possible—that is, that the turkeys transmit these viruses to other hosts, particularly humans in close contact with them. The infection of the laboratory technician by one of the turkey isolates establishes the potential for such an event. Whether such transmission occurs in nature is not known; however, in cases of human infection with swine viruses, epidemiologists should consider turkeys as well as pigs as a potential source.

V. S. HINSHAW
R. G. WEBSTER
W. J. BEAN
Division of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, Tennessee 38101
J. DOWNIE
Viral Products, Parkville, Victoria 3052, Australia
D. A. SENNE
Diagnostic Virology Laboratory, National Veterinary Service Laboratories, Ames, Iowa 50010

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Mycoplasma-Like Organisms: Occurrence with the Larvae and Adults of a Marine Bryozoan

Abstract. *Larvae and adults of the marine bryozoan* Watersipora cucullata *invariably possess numerous extracellular mycoplasma-like organisms. Mesodermally encapsulated groups of these atypical bacteria occur in the visceral coeloms of all colony members. In contrast, thousands of the symbionts are externally attached to each larva along a unique superficial groove; the microorganisms are internalized during the complex metamorphosis, thus inoculating the incipient colony. The consequences to the bryozoan of this association are not known.*

Symbiotic associations of prokaryotes with the digestive tracts of various marine invertebrates are widespread in occurrence. Most of these associations involve Gram-positive or Gram-negative eubacteria. Comparatively little is known, however, of the diversity and significance of nonpathogenic associations of prokaryotes with other organs of marine invertebrates (*1*). We report here the occurrence of mycoplasma-like organisms with the larvae and adults of a marine bryozoan *Watersipora cucullata* (*2*) and describe the transfer of these symbionts from the larva to the progenitor and successive, asexually produced individuals of the adult colony. Mycoplasma-like organisms are well known from vertebrates, insects, and plants (*3*) but have seldom been observed in marine invertebrates (*4*).

Colonies of *W. cucullata* were collected in April 1969 and from March through July 1982 from four localities along the southern California coast (*5*). Adults and larvae have many pleomorphic prokaryotic cells that lack discernible cell walls (*6*). These cells vary in size, usually from 3 to 6 μm in diameter, but some are as large as 14 μm. Their shape, as determined from reconstruction of 1-μm sections, examination of living cells, and by scanning electron microscopy, is globular with one or several large lobes (Fig. 1, A and C). The finely granular cytoplasm is virtually free of organelles, although the cells frequently contain a central, spherical, electron-opaque body that may be ribosomal. The cells lack a discrete nucleoid region, but wisps of DNA are seen in some sections. The entire cells fluoresce intensely in living and Formalin-fixed preparations after staining with DAPI (Fig. 1B), indicating the presence of DNA (*7*). The plasma membranes lack a discernible extracellular coat and, frequently, occur in apposition to the membranes of adjacent cells. Identification of the symbiont can only be tentative at this time because initial attempts to isolate and culture the cells have not been completed. Morphological evidence suggests that the cells are mycoplasms or L-form bacteria. We refer to them here as mycoplasma-like organisms (MLO's).

In *W. cucullata* larvae, MLO's are found in a dense stratum lining the floor of a specialized latitudinal groove on the surface of the larva (Fig. 1C). The transformation from larva to adult results from a complicated sequence of morphogenetic movements that occurs in all known bryozoans (*8*, p. 91). The groove is situated between the corona (the ciliated larval locomotory organ) and the pallial sinus. The groove is approximately 22 μm deep and 17 μm high and encircles the larva except in the anterior midline where it is interrupted for about 20 degrees. The floor of the groove is formed by the most aboral portions of 28 to 30 of the 32 coronal cells. The MLO's are attached to elongate microvilli that project into the groove. Each larva may have hundreds to several thousands of the prokaryotes. The remaining portions of the groove are formed by a ring of approximately 200 biciliated, supracoronal cells which collectively form a flange that overhangs the groove as a roof. Although most components of the larval anatomy of this bryozoan can be considered homologous with structures in other bryozoan larvae (*8*, p. 97), the groove and flange have no known counterparts. They appear to be specializations of this larva and thus facilitate the transport of the symbiotic microorganisms from one adult generation to the next.