

Immunity against avian influenza viruses emerging during an outbreak



Peter R. Woolcock^A, Jinling Li^B, Carol J. Cardona^B

^ACalifornia Animal Health and Food Safety Laboratory System – Fresno Lab, 2789 S Orange Avenue, Fresno, CA 93725

^BDepartment of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, Davis, CA 95616

Introduction

Avian influenza virus (AIV) had never infected commercial egg laying chickens in California prior to February 2000. Since then the virus persisted in the poultry population in California until 2005. All of the AIVs isolated were subtyped as H6N2 and determined to be of low pathogenicity for chickens. However in egg production flocks, the virus was the cause of increased mortality and a decrease in egg production. Lesions associated with this virus were unusual and included a characteristic yolk peritonitis. Three different genotypes of the H6N2 AIV were detected in California by 2001 and at least two of the viral genotypes were detected as late as 2003 (Woolcock and Suarez, unpublished). California is a large state with a diverse poultry population including egg layers, broilers, turkeys and other at-risk species such as quail and ducks.

In 2000, vaccination of a chicken layer flock against AI was permitted for the first time in California. A heterologous H6N8 vaccine was used because it was immediately available. A pullet vaccination model was used, where each new flock added to a premises with a previous infection was vaccinated. An H6N2 vaccine was used once available.

Cross neutralization tests were performed to gain more information on the antigenic status of the isolates. The sequence of each hemagglutinin gene was also compared to determine the differences in nucleotide and amino acid sequences of the three virus isolates. These sequences were compared with the sequences of five other H6N2 virus isolates from the same disease outbreak.

Materials and Methods

Viruses. Isolate A, iA: A/chicken/California/1002/2000 (H6N2) was isolated 14 March 2000 from 58-wk-old layers showing decreased egg production, respiratory signs of disease, and increased mortality. It was isolated from premises where the first vaccination was permitted and was genotype B based on the NS (non-structural) gene allele present. This isolate was obtained before the flock was vaccinated. Isolate B, iB: A/chicken/California/1255/2002 (H6N2) was isolated 30 January 2002 from a sentinel chicken showing acute fibrous peritonitis in a flock of vaccinated 118-wk-old layers. This flock was not vaccinated. Isolate C, iC: A/chicken/California/1963/2002 (H6N2) was isolated 15 February 2002 from 86-wk-old layers showing yolk and fibrous peritonitis. This flock was vaccinated. The isolate was obtained from the same premises as isolate A, and was determined to be a different genotype (genotype A), on the basis of the NS gene allele (D. Suarez, pers. comm.).

Cross-neutralization. Antisera were tested for the ability to neutralize or cross-react with the H6N2 virus isolates used to produce rabbit antisera following standard methods. The percentage plaque number reduction is calculated by: reduction % = (pfu/mL with naïve serum – pfu/mL with antiserum) / pfu/mL with naïve serum.

RT-PCR and sequencing. Viral RNA was extracted using the Viral RNA extraction kit according to the manufacturer's instructions (In Vitrogen, Carlsbad, CA). RT-PCR was done according to previously published protocols. RT-PCR amplicons were directly sequenced (Davis Sequencing, Inc., Davis, CA).

Amino acid changes. Sequences used in this study are listed in Table 1.

Strain name	Accession number	reference
A/chicken/CA/431/2000	AF457704	9, 10
A/chicken/CA/650/2000	AF474031	9, 10
A/chicken/CA/1002/2000	AF474032	9, 10
A/chicken/CA/139/2001	AF474033	9, 10
A/chicken/CA/1316/2001	AF474034	9, 10
A/chicken/CA/9420/2001	AF474038	9, 10
A/chicken/CA/1255/2002	FJ872078	This study
A/chicken/CA/1963/2002	FJ872079	This study

Results

Cross neutralization tests. See Table 2

RT-PCR and sequencing and amino acid changes. Sequencing of the HA gene of the 3 viruses revealed that

Antisera	AIV	iA	iB	iC
aA	1002/00	100	83.8	1.6
aB	1255/02	24.1	100	66.8
aC	1963/02	35.2	91.8	11.9

there were nucleotide differences that affected the amino acid type at 18 positions (62, 139, 142, 144, 145, 153, 156, 167, 170, 202, 213, 231, 282, 286, 327, 342, 382). Amino acid sequence identity of partial HA proteins of the three isolates is shown in Table 3. The aa sequence identity of partial HA proteins of the 3 isolates used in the cross neutralization study together with sequences of five published H6N2 viruses from the outbreak are shown in Table 4.

	ck/CA/1002/2000	ck/CA/1255/2002	ck/CA/1963/2002
ck/CA/1002/2000	100%	95.57%	95.83%
ck/CA/1255/2002	--	100%	99.22%
ck/CA/1963/2002	--	--	100%

AA position	62	114	125	139	142	144	145	149	153	156	167	170	202	213	223	231	282	286	327	342	382	
notes	AIV																					
	431/00	N	I	E	A	D	S	S	R	Y	D	I	K	D	R	T	A	V	N	G	E	R
	650/00	D	I	E	A	D	S	S	R	Y	D	I	K	D	R	T	A	V	N	G	E	R
premises 1	1002/00	N	I	E	A	D	S	S	R	Y	D	I	K	D	R	T	A	V	N	G	E	R
	139/01	S	I	E	S	N	N	N	R	D	G	T	N	N	R	N	S	V	N	E	A	R
	1316/01	S	I	G	S	N	N	N	R	D	G	T	N	N	R	N	S	V	N	E	A	R
	9420/01	S	M	E	S	N	N	N	I	D	G	T	N	N	R	N	S	V	N	E	A	R
premises 2	1255/02	S	I	E	S	N	N	N	R	D	G	T	N	N	K	N	S	I	N	E	A	K
premises 1	1963/02	S	I	E	S	N	N	N	R	D	G	T	N	N	R	N	S	V	D	E	A	R

Discussion

Neutralization results show that these isolates are different, with less cross neutralization than expected. These findings suggest significant antigenic change among these isolates and that they were not equally antigenic (antiserum to isolate C had very little capacity to neutralize the homologous isolate; 11.9%). Interestingly, isolates A and C are from the same premises but there is little cross neutralization between them. Also, there is more similarity in neutralization profiles between viruses isolated in the same year (but more than 100 miles apart Table 4) than between isolates from the same location.

The viruses studied differed by only a few amino acids in the HA gene. Interestingly, the viruses from the same year (B and C) were very similar to each other while the viruses from the same premises diverged (A and C). These findings, along with the neutralization profiles for these viruses would suggest that viruses A and C represented separate introductions on to the same premises and that virus B and virus C shared the same ancestor. This is further reinforced by the aa sequences of the HA protein of the 5 other viruses investigated Table 4. It can be seen that the 2000 isolates are distinct from the 2001 and 2002 isolates by 14 amino acids.

The isolates compared in this study were isolated from commercial chicken flocks in Southern California in 2000 and 2002. They varied in their abilities to neutralize themselves and each other. In general, many California egg production flocks were vaccinated including flocks on the premises where isolates A and C were recovered. In fact, flock C was vaccinated between 15 and 20 weeks of age. We know that over time in commercial flocks, immunity from vaccination wanes allowing for new infections. These studies suggest that there was little cross protective immunity in the flock even shortly after vaccination due to antigenic drift. It is likely that flock C had little vaccinal immunity at the time of infection (90 weeks later) but also that the vaccine strain had little protective capacity due to antigenic drift.

The vaccination model used in California egg production flocks was focused on vaccinating new susceptible birds coming onto the farm, i.e., pullets. This type of vaccination program was cost effective for the producers but had to be coupled with strict biosecurity to prevent new introductions of virus into vaccinated flocks. In some cases, the combination of vaccination and biosecurity worked. But, in an outbreak, there are many cases and it is the collective outcome that determines whether a disease is controlled or not. The vaccination model used in California presented the opportunity for failures. It is very likely that one of the drivers of the antigenic drift observed in these studies was impartial and poorly monitored vaccination on at least some of the farms in the region. Improper vaccination has been reported to drive antigenic drift in other studies. We do not know specifically where and when the ancestor of isolates B and C arose, but it arose from the collection of farms that were partially vaccinating without sufficient biosecurity to prevent new infections. Although this vaccination model can work, it did not work collectively in Southern California and the result was the spread of H6N2 (A/chicken/California/139/01) to new populations in Northern California.

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