

Avian Leukosis Virus Subgroup J in Layer Chickens, China

To the Editor: In recent years, cases of avian leukosis virus subgroup J (ALV-J) infection and tumors in commercial layer chickens and breeders of egg-type chickens have been emerging in the People's Republic of China. ALV-J was first isolated from meat-type chickens with myeloid leukosis in 1988. Although egg-type chickens have been experimentally infected with ALV-J to induce tumors (1), field cases of ALV-J infection and tumors in commercial layer chickens were not found worldwide until 2004 (2).

ALV-J has recently been found to have induced various tumors and caused production problems in commercial layer flocks and local chicken breeds in China (2,3). Many field cases of ALV-J infection and tumors have occurred in 15- to 29-week-old egg-type chickens in several provinces. Affected flocks had dramatically reduced egg production and hemorrhage in the skin surrounding the phalanges and feather follicles. Some birds had gray-white nodules in the liver, spleen, or kidneys, and liver and spleen were enlarged up to several times their normal size. Morbidity rates for some flocks reached 60%, and mortality rates for some flocks were >20%. Clinical samples from livers, spleens, whole blood, and tumors were collected from chickens in different provinces and sent for laboratory diagnosis. Results showed that the predominant virus in the samples was ALV-J.

During 2007–2009, we conducted an epidemiologic investigation of ALV in layer flocks in China. All virus isolation was performed in DF-1 cells. Briefly, 233 clinical samples were collected from 44 layer flocks in different provinces and used to inoculate subconfluent cell cultures containing Dulbecco modified essential medium

supplemented with 10% (vol/vol) fetal bovine serum and antimicrobial drugs. After a 7–9 day incubation period, the cells were frozen and thawed 3×. A group-specific antigen-capture ELISA was used to identify ALV. After proviral DNA was extracted directly from infected cell culture or tumors, PCR with strain-specific primers was used to detect ALV-A, ALV-B, or ALV-J (4).

Of these samples, 150 (64.4%) were ALV-J positive, 28 (12.1%) were ALV-A positive, and 8 (3.4%) were ALV-B positive. Phylogenetic analysis showed an 87.3%–98.2% aa sequence identity of *env* genes in all ALV isolates compared with the HPRS-103 strain (5). All isolates had complete repeated transmembrane deletion and partial direct repeat–1 deletion but contained an intact E element. A mutation was found in the enhancer and promoter region of the U3 region in the 3' long terminal repeat; this mutation is not found in ALV-J isolated from broiler chickens (6).

The newly isolated ALV-J strain from layer chickens was used to examine the pathogenicity in 1-day-old White Leghorn specific pathogen-free chicks soon after hatching in separate incubators and rooms in the experimental animal house facilities at Harbin Veterinary Research Institute, Harbin, China. The chicks were inoculated intraabdominally with a 1,000-unit 50% tissue-culture infective dose of ALV-J propagated in the DF-1 cells. Blood samples were collected to check for viremia at 10 weeks of age. Experimental birds were reared until 27–30 weeks of age.

Prolonged viremia developed in 15 (50%) of 30 chicks; hemangiomas developed in the skin surrounding phalanges and in the liver of 3 (10%); and myeloid leukosis, detected by gross or histologic examination, developed in 10 (30.3%). A previous study showed that meat-type birds infected with ALV-J retained a high level of viremia over their lifetime (7) but that layer chickens cleared the infection within

a few weeks. Our study demonstrated that ALV-J infection can cause disease in layer chickens and can induce tumors and long-lasting viremia. For this reason, disease caused by ALV-J in layer chickens in China should be further investigated.

Because ALV-J is vertically transmitted from dam to progeny by the embryo, it represents a potential threat for humans who receive vaccines that are produced in chicken embryonic fibroblasts or embryonated eggs (e.g., yellow fever vaccine and measles and mumps vaccine) (8). An effective vaccine against ALV is not available. Eradication of ALV-J has been difficult because of substantial genetic and antigenic variation among ALV-J isolates as well as high levels of vertical and horizontal transmission (9,10). Therefore, effective prevention and elimination measures should be developed as soon as possible.

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Healthcare Worker Acceptance of Pandemic (H1N1) 2009 Vaccination, Morocco

To the Editor: In Morocco, the first case of pandemic (H1N1) 2009 was diagnosed on June 12, 2009 (1). Because a main determinant of public immunization success is healthcare workers' support and recommendations and because little is known about such with regard to pandemic (H1N1) 2009 vaccination in Morocco, our aim was to document healthcare workers' knowledge, attitudes, practices, and acceptance of pandemic (H1N1) 2009 vaccination in Morocco.

From January 15 through February 28, 2010, a structured, self-administered, anonymous questionnaire was distributed to a convenience sample of 1,332 healthcare workers in 5 public hospitals in Rabat, Morocco. Completed questionnaires were analyzed by using SPSS version 10.0 (SPSS, Chicago, IL, USA). The 1,002 responses gave a response rate of 75% ($\approx 17\%$ of the entire staff of the University Hospital of Rabat).

We found that the hospital staff had acquired basic knowledge about transmission and prevention of the pandemic (H1N1) 2009 virus. Responses indicated that 218 (22%) study participants had accepted vaccination (i.e., had been vaccinated) against this virus. Markedly more healthcare workers in Morocco were undervaccinated than were those in the United States; by mid-January 2010, estimated vaccination coverage among healthcare workers was 37.1% (2). Some evidence indicates that willingness of healthcare workers to be vaccinated with the new vaccine is poor: 48.0% in Hong Kong Special Administrative Region, People's Republic of China (3) and 22.3% in the United States (4). Vaccination coverage was significantly higher for those 20–30 years of age

than for those in other age groups ($p = 0.001$). The analysis by occupational category showed significantly higher coverage for paramedical staff (26%) than for physicians and pharmacists (19%) ($p < 0.01$). The main causes for this reluctance were fear of adverse effects, concerns about the new adjuvant used, the short duration of clinical trials, and influence of the media.

The low acceptance rate of vaccination for pandemic (H1N1) 2009 among healthcare workers in Morocco is alarming because they serve as an example for their patients and the public. Vaccination is needed to keep the healthcare system operating at maximum capacity during a pandemic. The following factors appear to play a major role in acceptance: accessibility of the vaccine within the service; free vaccine; and a display explaining vaccination's benefits, protective value, and risk for adverse effects (5,6). Policy makers could use our findings to improve the vaccination strategy for healthcare workers in future vaccination campaigns.

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