COMPARISON OF REAL-TIME REVERSE TRANSCRIPTION–PCR AND VIRUS ISOLATION FOR ESTIMATING PREVALENCE OF AVIAN INFLUENZA VIRUS IN HUNTER-HARVESTED WILD BIRDS AT WATERFOWL WINTERING GROUNDS ALONG THE TEXAS MID–GULF COAST (2005–2006 THROUGH 2008–2009)

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Important Findings

Historically, virus isolation has been the method of choice for conducting surveillance for avian influenza virus (AIV) in avian species. More recently, real-time reverse transcription–polymerase chain reaction (RRT-PCR) has become the primary screening method. The goal of this study was to determine how two testing methods (virus isolation and RRT-PCR) affect AIV prevalence estimation, particularly in an understudied, low-prevalence region—the waterfowl wintering grounds along the Texas mid–Gulf Coast. The data indicate that screening samples by RRT-PCR followed by virus isolation only on RRT-PCR–positive samples provides a reasonable means to generate prevalence estimates close to the true prevalence as determined by virus isolation. The study also confirms the low prevalence of AIV in waterfowl wintering grounds along the Texas mid–Gulf Coast and demonstrated little variation in prevalence among months during the four hunting seasons sampled.

Significance of Findings

As with any study, the most important aspect to consider when deciding which detection method to use (i.e., RRT-PCR and/or virus isolation) is the goal of sampling (i.e., process more samples or obtain all possible isolates). Over the 4-yr course of this study, researchers performed virus isolation on 4953 samples and RRT-PCR on 6824 samples, resulting in 138 AIV isolates, of which 2 were obtained from RRT-PCR–negative samples. Thus, if the focus of the surveillance program is processing as many samples as possible, screening by RRT-PCR, and then performing virus isolation only on RRT-PCR–positive samples, provides a reasonable means to process a large number of samples and generate prevalence estimates quite similar to the true prevalence as determined by virus isolation.

Additional Information

As concerns over the spread of highly pathogenic avian influenza (HPAI) H5N1 viruses have increased, surveillance programs have been implemented worldwide. The primary method utilized internationally for screening samples for AIV is RRT-PCR for the matrix gene, a highly conserved gene in type A influenza viruses. Virus isolation has also been used in some cases, primarily for confirmation in the event of positive RRT-PCR results. Additionally, within many of these surveillance programs, any sample positive for type A influenza virus is further screened by RRT-PCR specific for H5 and H7 subtypes, the two subtypes most commonly associated with losses in poultry and classified as notifiable AIV by the World Organization for Animal Health (OIE).

Prior to the implementation of surveillance programs focused on identifying HPAI H5N1, most studies reporting prevalence of influenza viruses in North America were conducted during the late summer to early fall when premigration staging occurs, and therefore were concentrated in Alaska, Canada, the upper midwestern United States, and the northeastern United States. Few studies involved waterfowl on their wintering grounds or nonmigratory waterfowl during winter; this is particularly true along the Gulf Coast, where such studies were limited to just a few waterfowl species and time points. Additionally, in these studies, virus isolation was the primary method for screening samples and identifying AIV, hence prevalence estimates were based on these results.

COMPARACIÓN DE PCR-TRANSCRIPCIÓN INVERSA EN TIEMPO REAL Y AISLAMIENTO VIRAL PARA ESTIMAR LA PREVALENCIA DEL VIRUS DE INFLUENZA AVIAR EN AVES SILVESTRES ACUÁTICAS CAZADAS Y RECOLECTADAS EN TERRENOS PARA INVERNAR A LO LARGO DE LA COSTA MEDIA DEL GOLFO EN TEXAS (2005–2006 HASTA 2008–2009)

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Hallazgos Importantes

Históricamente, el aislamiento viral ha sido el método de elección para conducir la vigilancia del virus de influenza aviar (AIV, por sus siglas en inglés) en especies aviares. Más recientemente, la reacción en cadena de polimerasas-transcripción inversa en tiempo real (RRT-PCR, por sus siglas en inglés) se ha convertido en el principal método

de monitoreo. El objetivo de este estudio fue determinar cómo probando dos métodos de prueba (aislamiento viral y RRT-PCR) se afecta la estimación de la prevalencia del AIV, particularmente en una región subestudiada de baja prevalencia—los terrenos para invernar de las aves acuáticas a lo largo de la Costa Media del Golfo en Texas. Los datos indican que las muestras de monitoreo por RRT-PCR seguidos por el aislamiento viral sólo en muestras positivas de RRT-PCR provee una media razonable para generar cálculos de prevalencia cercanos a la verdadera, tal y como se determina por medio del aislamiento viral. El estudio también confirmó la baja