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Research Note—

Avian Influenza Virus Investigation in Wild Bobwhite Quail from Texas

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SUMMARY. The objective of this study was to determine the prevalence of avian influenza viruses (AIV) in bobwhite quail (*Colinus virginianus*) populations from the rolling plains of Texas, U. S. A. A total of 1320 swab samples (652 tracheal swabs and 668 cloacal swabs) and 44 serum samples were collected from wild-captured or hunter-harvested bobwhite quail from November 2009 to April 2011 at the Rolling Planes Quail Research Ranch, Fisher County, Texas, U. S. A. The presence of AIV in the swabs was determined by real-time reverse-transcription–PCR (rRT-PCR) and all samples positive or suspicious by rRT-PCR were further processed for virus isolation in embryonated chicken eggs. A total of 18 (1.4%) swab samples tested positive for AIV by rRT-PCR (cycle threshold [Ct] values <35): 13 cloacal swabs (1.9%) and 5 tracheal swabs (0.8%). In addition, 100 (7.6%) swab samples were considered suspicious (Ct values 35.1–40): 69 cloacal swabs (10.3%) and 31 tracheal swabs (4.7%). No virus was isolated from any of the rRT-PCR–positive or suspicious samples tested. Additionally, 44 serum samples were screened for AIV antibodies and were negative. The results presented here indicate low prevalence of AIV in wild populations of bobwhite quail.

RESUMEN. *Nota de Investigación*—Investigación sobre el virus de la influenza aviar en codornices norteañas silvestres del Estado de Texas.

El objetivo de este estudio fue determinar la prevalencia de virus de influenza aviar (AIV) en las poblaciones de codornices norteañas (*Colinus virginianus*) de las llanuras de Texas, en los Estados Unidos. Un total de 1320 muestras de hisopos (652 hisopos traqueales y 668 hisopos cloacales) y 44 muestras de suero fueron recolectadas de codornices silvestres capturadas o cazadas desde noviembre del año 2009 a abril de año 2011 en el Rancho de Investigación de Codornices Rolling Planes, en el condado de Fisher en Texas. Se determinó la presencia del virus de la influenza aviar en los hisopos mediante un método de transcripción reversa y reacción en cadena de la polimerasa en tiempo real (rRT-PCR) y todas las muestras positivas o sospechosas por rRT-PCR se procesaron para el aislamiento del virus en huevos embrionados de pollo. Un total de 18 muestras de hisopos (1.4%) resultaron positivas para el virus de influenza aviar por rRT-PCR (valores de ciclo umbral [CT] menores a 35): 13 hisopos cloacales (1.9%) y 5 hisopos traqueales (0.8%). Además, 100 muestras de hisopos (7.6%) se consideraron sospechosas (valores de Ct de 35.1–40): 69 hisopos cloacales (10.3%) y 31 hisopos traqueales (4.7%). No se aislaron virus de ninguna de las muestras positivas o sospechosas analizadas por rRT-PCR. Además, 44 muestras de suero fueron examinadas para detectar anticuerpos contra el virus de influenza aviar, las cuales fueron negativas. Los resultados presentados aquí indican una baja prevalencia de virus de influenza aviar en las poblaciones silvestres de codorniz.

Key words: bobwhite quail, avian influenza, Texas

Abbreviations: AI = avian influenza; AIV = avian influenza virus; ELISA = enzyme-linked immunosorbent assay; rRT-PCR = real-time reverse-transcription–polymerase chain reaction

Wild waterfowl and shore birds are the natural reservoirs of all avian influenza viruses (AIV) (18); however, these viruses are occasionally transmitted to terrestrial birds where they can cause varying degrees of disease from asymptomatic infections to severe systemic disease (16). Highly pathogenic (12) and low pathogenic (1,2,15) AIVs of different subtypes have been isolated from commercial quail populations throughout Asia and North America (10,20) and more recently from commercial bobwhite quail (*Colinus virginianus*) in Egypt (4). Additionally, numerous reports have shown that quail are susceptible to infection with many different AIVs (3,7,8,19). However, to our knowledge, the prevalence of AIV infections in wild quail populations has not been investigated.

The objective of this study was to determine the prevalence of AIV in free-roaming quail populations from the rolling plains of Texas.

MATERIALS AND METHODS

Sample collection. Both cloacal and tracheal swab samples were collected for AIV testing by trained personnel from either hunter-harvested or live-trapped wild bobwhite quail at the Rolling Plains Quail Research Ranch in Fisher County, Texas, U. S. A. The sex and age of the birds was determined by trained personnel using feathering characteristics. For age, the absence of buff-tipped coverts indicated the bird was at least 1 yr of age and was thus classified as adult; birds less than 1 yr of age were classified as juvenile (11). Swab samples were collected, processed, and tested as previously described (5,6) with a few exceptions, as noted. Samples were transported from the field on ice and stored at –20 C until shipped overnight to the laboratory. If the samples arrived thawed, samples were vortexed, centrifuged at 1,500 × g for 10 min, and 100 μl of supernatant was dispensed into 96-well plates for RNA isolation. If the samples arrived frozen, the samples were thawed and

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Table 1. Avian influenza matrix rRT-PCR results by age, season, and sample type for fall 2009, 2010 and spring 2010, 2011.

Result ^A	Adult ^B (%)			Juvenile (%)			Male (%)			Female (%)		
	Positive	Suspicious	Negative	Total	Positive	Suspicious	Negative	Total	Positive	Suspicious	Negative	Total
Cloacal swabs												
Fall 2009	0	0	82 (100)	82	0	4 (2.6)	152 (97.4)	156	0	2 (1.5)	131 (98.5)	133
Spring 2010	0	5 (15.6)	27 (84.4)	32	2 (3.4)	4 (6.8)	53 (89.8)	59	0	4 (8.2)	45 (91.8)	49
Fall 2010	1 (3.8)	4 (15.4)	21 (80.8)	26	2 (1.8)	16 (14.5)	92 (83.6)	110	3 (4.6)	9 (13.8)	53 (81.5)	65
Spring 2011	4 (9.2)	12 (16)	59 (78.7)	75	4 (3.2)	24 (19.2)	97 (77.6)	125	3 (2.9)	19 (18.2)	83 (79.0)	105
Total	5 (2.3)	21 (9.8)	189 (87.9)	215	8 (1.8)	48 (10.7)	394 (87.6)	450	6 (1.7)	34 (9.7)	312 (88.6)	352
Tracheal swabs												
Fall 2009	0	0	82 (100)	82	1 (0.6)	1 (0.6)	155 (98.7)	157	1 (0.8)	0	132 (99.2)	133
Spring 2010	0	1 (3.1)	31 (96.9)	32	0	7 (12.1)	51 (87.9)	58	0	5 (10.0)	45 (90.0)	50
Fall 2010	2 (8.7)	1 (4.3)	20 (87.0)	23	1 (1.0)	2 (2.0)	98 (97.0)	101	1 (1.6)	2 (3.3)	58 (95.1)	61
Spring 2011	0	8 (11.0)	65 (89.0)	73	1 (0.8)	11 (9.0)	111 (91.0)	122	0	9 (8.7)	94 (91.3)	103
Total	2 (1.0)	10 (4.8)	198 (94.3)	210	3 (0.7)	21 (4.8)	415 (94.7)	438	2 (0.6)	16 (4.6)	329 (94.8)	347

^APositive = Ct <35.0, suspicious = Ct 35.1–40.0, negative = Ct >40.

^BMature feathering >1 year = adult.

then processed as described above. All samples and 96-well plates were frozen at -80 C until processed for virus isolation or RNA isolation.

RNA extraction, real-time reverse transcription-PCR (rRT-PCR) and RT-PCR for sequencing. RNA was extracted and rRT-PCR for the avian influenza (AI) matrix gene was performed as previously described (5,14). Virus isolation was performed on all AI-matrix rRT-PCR-positive (cycle threshold [Ct] value <35) and suspicious (Ct values 35.1–40) samples as previously described (6,17).

Enzyme-linked immunosorbent assay (ELISA) for detection of AIV antibodies. Forty-four serum samples from live-captured quail were collected from the jugular vein by trained personnel from the United States Department of Agriculture, Animal and Plant Health Inspection Service – Wildlife Services, frozen at -20 C, and shipped overnight to the laboratory. A commercial ELISA kit (IDEXX FlockChek™, AI MultiS-Screen Ab test, Westbrook, ME) was used to screen for the presence of antibody to AIV following manufacturer’s protocol.

Statistical analysis. Data were analyzed using Intercooled Stata version 10 (Stata Corp., College Station, TX).

RESULTS AND DISCUSSION

A total of 1320 swab samples (652 cloacal and 668 tracheal swab samples) were collected from 688 individual quail. Of the cloacal swabs collected, 52.7% were from male birds, 44.6% were from females, and sex was not determined for 2.7%. Of the tracheal swabs collected, 53.2% and 44.5% were from males and females, respectively, with 2.3% from birds for which sex was not determined. The majority of samples were collected from juvenile birds, 450 (67.4%) cloacal swabs and 439 (67.3%) tracheal swabs, and age was not determined for less than 0.5% of the tracheal and cloacal swab samples.

Eighteen (1.4%) swab samples tested positive for AIV by rRT-PCR (Ct values <35): 13 cloacal swabs (1.9%) and 5 tracheal swabs (0.8%). One hundred (7.6%) swab samples were considered suspicious (Ct values 35.1–40): 69 cloacal swabs (10.3%) and 31 tracheal swabs (4.7%). No virus was isolated from any of the rRT-PCR-positive or suspicious samples tested. More cloacal swab samples were suspicious or positive for AIV in the Spring of 2011 as compared to previous seasons. No significant differences were observed between adults and juveniles or between males and females (Table 1).

Presence of AIV genomic material was confirmed in selected samples by RT-PCR amplification using primers specific to the NS and NP gene segments, followed by sequencing (data not shown). No AIV antibodies were detected in 44 quail serum samples tested.

To our knowledge, this is the first study to specifically examine AIV in the wild bobwhite quail population. Others have tested quail for AIV, but as opportunistic and not targeted sampling; they also observed a few positive samples by rRT-PCR and no isolates were obtained (13). The lack of successful virus isolation in our study is not particularly surprising in that sample handling was not optimal. The samples were collected in the field with concerted effort to keep them chilled until transported back to the office where they were stored at -20 C until shipped overnight to the laboratory. The samples were stored for various lengths of time at -20 C prior to shipping and often arrived at the laboratory having thawed in transit. The varying lengths of time stored at -20 C should not have been an issue, because swab samples can be stored for 1 wk to months and maintain sample integrity (9). The samples arriving thawed would be a problem because freeze-thaw cycles can be detrimental. However, the low AIV prevalence detected by rRT-PCR, and the lack of virus isolation, could also be due to minimal exposure of the

quail population to AIV as supported by the serologic data presented in this study.

Our results support the fact that there continues to be little data to suggest that terrestrial birds such as Galliformes play a major role in the natural history of AIV. However, additional surveillance, both serologic and virologic, is needed to confirm these findings.

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