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Research Note—

Detection of Avian Paramyxoviruses in Migratory and Resident Birds in the State of Rio de Janeiro, Brazil

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SUMMARY. Paramyxoviruses and avian influenza viruses are present worldwide, and wild birds are known natural reservoirs of these viruses. This study monitored the circulation of these viruses in migratory and resident coastal birds captured in the state of Rio de Janeiro, Brazil. In total, 494 birds were trapped, and their fecal samples were collected and inoculated into embryonated chicken eggs. The allantoic fluids were evaluated using a hemagglutination test and PCR amplification of the genes of the M and L proteins of influenza A virus and paramyxovirus, respectively. Avian paramyxovirus was detected in 5 (1.01%) of the birds. The majority of these viruses were isolated from migratory birds classified into the order Charadriiformes (families Scolopacidae and Charadriidae). Four samples were characterized as avian paramyxovirus serotype-2 (APMV-2) by a hemagglutination inhibition test. These results reinforce the importance of continuous surveillance of wild species in Brazil.

RESUMEN. *Nota de Investigación*—Detección de paramixovirus aviares en aves migratorias y residentes en el Estado de Rio de Janeiro, Brasil.

Los paramixovirus y los virus de la influenza aviar están distribuidos mundialmente y las aves silvestres sirven como reservorios naturales de estos virus. En este estudio se analizó la circulación de estos virus en aves costeras migratorias y residentes que fueron capturadas en el Estado de Rio de Janeiro, Brasil. Un total de 494 aves fueron capturadas y muestras fecales de estas aves fueron recolectadas e inoculadas en huevos embrionados de pollo. Se analizaron los fluidos alantoideos de los huevos inoculados mediante pruebas de hemaglutinación y amplificación por PCR de los genes que codifican las proteínas M y L del virus de la influenza y paramixovirus, respectivamente. Se detectaron paramixovirus aviares en cinco muestras (1.01%) de las aves. El mayor número de estos virus fueron aislados de aves migratorias clasificadas dentro del orden Charadriformes (familias Scopolacidae y Charadridae). Cuatro muestras se caracterizaron como paramixovirus serotipo-2 (APMV-2) mediante la prueba de inhibición de la hemaglutinación. Estos resultados refuerzan la importancia de la vigilancia continua de las especies silvestres en Brasil.

Key words: avian paramyxovirus-2, migratory birds, Brazil

Abbreviations: AIVs = avian influenza viruses; APMV = avian paramyxovirus; CEMAVE = Centro Nacional de Pesquisa e Conservação de Aves Silvestres; FAPERJ = Fundação Carlos Chagas Filho de Amparo á Pesquisa do Estado do Rio de Janeiro; HAU = hemagglutinating unit; HI = hemagglutination inhibition; IBAMA = Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis; NDV = Newcastle disease virus; PBS = phosphate-buffered saline; TBE = Tris-borate-EDTA buffer

The viruses that infect wild animals have become increasingly important throughout the world in recent decades, substantially impacting human health, agricultural production, wildlife-based economies, and wildlife conservation (5). Wild birds play a major role in spreading paramyxoviruses and orthomyxoviruses, which have caused diseases in humans and animals for centuries (2,11,25). Avian influenza A viruses (AIVs), classified into the family *Orthomyxoviridae*, are pleomorphic and have enveloped structures, and their capsids contain segmented negative-sense, single-stranded RNA genomes (23). Avian paramyxoviruses (APMV1 to APMV11), part of the genus *Avulavirus*, family *Paramyxoviridae*, exhibit many characteristics similar to influenza viruses but present a nonsegmented RNA genome (7,16). Migratory wild birds have a central role as reservoirs of AIVs and APMVs in nature, spreading them to other species (1,2,21). Because the Southern Hemisphere receives 73 species of migratory birds from the north annually (32), there is a risk of the introduction of these viruses, mainly between September and May, when these birds visit Brazil (18). In the United States, the presence of antibodies to APMV1, APMV2, APMV3, APMV4, APMV6, APMV7, APMV8, and APMV9 in the sera of 100 commercial, layer-type and broiler-type chicken flocks analyzed on a random basis has been observed (33).

Although Newcastle disease virus (NDV) is considered to be a pathogen of most avian species (15,16), NDV circulation among migratory waterfowl has not been frequently studied among shorebirds in Brazil. Governmental prophylactic measures have already ensured that Brazil is considered free of virulent Newcastle disease virus; however, the huge territory of the country contains a

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great diversity of wildlife, including wild migratory birds (6). These birds may be natural reservoirs or carriers of NDV because many migratory birds come from regions where these viruses are endemic. After the recognition of Brazil as being a virulent-NDV-strain-free country for commercial poultry, the introduction of continuous surveillance has permitted the accumulation of a large amount of data on the circulation of paramyxoviruses, mainly NDV. Previous data (22) revealed seropositivity for NDV among Brazilian asymptomatic birds of commercial poultry farms (28.8%) and detected only low-pathogenicity NDV strains, confirming the maintenance of the velogenic-NDV-free status for Brazil.

To investigate the circulation of avian paramyxoviruses and influenza A viruses among wild birds, we captured migratory and resident birds from the northeast coast of the state of Rio de Janeiro, Brazil, in spring. Serologic and molecular assays have already shown that APMVs circulate in migratory bird species on the eastern coast and Amazon regions of Brazil, mainly in the Charadriidae and Scolopacidae families (30). In this work, we collected samples in the northeast coast of the state of Rio de Janeiro, where there is an important complex of lakes where migratory bird species, mainly from Northern Hemisphere locations such as Canada, the lower U. S. A. and Alaska, feed and rest. These particular geographic characteristics favor the implementation of a virus surveillance program of migratory birds.

MATERIALS AND METHODS

Specimens. In total, 494 fecal samples were obtained from individual birds by trapping them in standard mist nets from November 2004 to February 2009 at Mangue da Carapeba–Farol de São Tomé, Campos dos Goytacazes city, Rio de Janeiro (22°0.5′S; 41°0.7′W). The collection was authorized by Centro Nacional de Pesquisa e Conservação de Aves Silvestres–Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (CEMAVE-IBAMA), number 1323-Cemave. Biometric analysis of the birds was performed in accordance with the rules of CEMAVE-IBAMA (8). Fecal samples were collected in phosphate-buffered saline (PBS)-glycerol transport medium (pH 7.2), diluted in the same medium, conserved on ice during the time of collection (approximately for 3–4 hr), and frozen at −20 C until the analysis. Before processing, the samples were diluted (10%) with PBS (pH 7.2) plus penicillin (500 IU) and amphotericin B (5 μg/ml) and centrifuged (600 × *g* for 15 min). The final supernatant was collected for further analysis.

Inoculation into embryonated eggs and hemagglutination test. For each fecal sample, 200-μl samples of the treated supernatants were inoculated in the allantoic cavity of specific-pathogen-free 9–11-day-old embryonated chicken eggs and incubated at 35–37 C for 48–72 hr for virus isolation and propagation. The allantoic fluid was harvested, and two additional passages into embryonated eggs were performed before a sample was considered negative by hemagglutination testing. After each passage, the samples were submitted to a hemagglutination test using chicken erythrocytes (0.5%), as previously described (19). The virus isolates were characterized using PCR and hemagglutination inhibition (HI) test.

RNA extraction and RT-PCR. Initially, the total RNA of each virus sample was extracted from the allantoic fluid using a QIAamp mini-kit (Qiagen, São Paulo, Brazil). Two-step RT-PCRs were performed as proposed by Ellis and Zambon (10). The influenza M (matrix protein) gene of influenza A viruses was amplified in the first nested-PCR with the primer pair AMP71F and AMP831R A. The second reaction used the primer pair AMP227F and AMP622R. For paramyxovirus detection, fragments of the L gene were amplified independently with the PAR-F2 and PAR-R primers (31). For specific NDV detection, the methodology described by Stäuber *et al.* (28) was followed, using the NCD3/4 primer set.

For reverse transcription (RT), 7-μl aliquots of RNA were added to an RT mixture containing 20 mM Tris-HCl (pH 8.4), 50 mM potassium chloride (KCl), 7.5 mM magnesium chloride ($MgCl_2$; Life Technologies, Grand Island, NY), 0.5 mM of each deoxynucleoside triphosphate, 1 pmol of reverse primer, RNase inhibitor (0.8 U/μl), and 1 U Moloney murine leukemia virus (M-MLV) reverse transcriptase (Gibco BRL, Carlsbad, CA). PCR was performed at 37 C for 60 min (RT), with specific test primers (50 pmol of each forward and reverse primer), reaction buffer (2.0 mM $MgSO_4$), 200 μM of each dNTP (Invitrogen), 5 μl of cDNA, and 1 U Taq polymerase (Life Technologies) in 50 μl of solution. The PCR mixtures were incubated at 94 C for 2 min, followed by 40 cycles at 94 C for 15 s, 48 cycles at 50 C for 30 s (or 54.9 C), 72 C for 30 s, and a final extension at 72 C for 7 min. The amplified products were visualized in agarose gels (1.5%) in Tris-borate-EDTA (TBE) buffer. Ethidium bromide was added to the gels.

Table 1. Distribution of birds sample and tested during November 2004 to February 2009. Birds were as assigned as migratory (m) or resident (r) by CEMAVE-IBAMA (7).

Family	Species	Number	%
Charadriidae	*Charadrius semipalmatus* (m)	141	28.54
	Charadrius colaris (r)	97	19.64
	Pluvialis squatarola (m)	5	1.01
	Vanellus chilensis (r)	1	0.2
Total		244	49.39
Scolopacidae	*Arenaria interpres* (m)	94	19.03
	Calidris fuscicollis (m)	36	7.29
	Tringa flavipes (m)	29	5.87
	Actitis macularia (m)	29	5.87
	Numenius phaeopus (m)	15	3.04
	Tringa melanoleuca (m)	7	1.42
	Gallinago paraguaiae (r)	1	0.2
Total		211	42.72
Spheniscidae	*Spheniscus magellanicus* (m)	14	2.83
Cuculidae	*Crotophaga ani* (r)	5	1.01
	Anas bahanensis (r)	4	0.81
Anatidae	*Anas boschas* (r)	3	0.61
	Dendrocygna viduata (r)	2	0.4
Total		9	1.82
Strigidae	*Speotyto cunicularia* (r)	2	0.4
Ardeidae	*Butorides striatus* (r)	2	0.4
Tytonidae	*Tito alba* (r)	1	0.2
Sternidae	*Sterna hirundo* (m)	1	0.2
Rostratulidae	*Nycticryphes semicollaris* (r)	1	0.2
Procellariidae	*Macronectes* (m)	1	0.2
Diomedeidae	*Thalassarche chlororhynchus (Diomedea)* (m)	1	0.2
Columbidae	*Columbina talpacoli* (r)	1	0.2
Caprimulgidae	*Chordeiles* sp. (m)	1	0.2
Total		494	99.97

Hemagglutination inhibition (HI) test. HI tests were performed as described in OIE (2012) using standard polyclonal chicken antisera (19) against different AIVs (H1N1, New Caledonia/20/99/2000; H2N2, CAP/2004; H3N8, Miami/63; H7N7, Prague/56), and APMV standard samples (APMV-1, NDV-La Sota; APMV-2, P/Ck/CA/Yucaipa/56; APMV-3, P/turkey/Wisconsin/68; APMV-6, DK/H.K./199/77; and APMV-7, Dove/TN/4/75) obtained from the United States Department of Agriculture National Veterinary Services Laboratories (Ames, IA) (16).

Electron microscopy. The identified samples were propagated in eggs. Their allantoic fluids were clarified at 2800 × *g*, concentrated at 65,000 × *g* for 1 hr, and purified in a sucrose gradient (20%–60%) by centrifugation at 80,000 × *g* for 2 hr. The purified viral suspensions were observed under a Morgani EM 268 electron microscope (Fei, Eindhoven, Netherlands) at 80 kV on a copper grid stained with 4% phosphotungstic acid, following the methodology established by Fonseca *et al.* (12).

RESULTS AND DISCUSSION

In our study, birds from 25 species (27) of 14 families (Table 1) were trapped to collect feces samples, ordered by incidence rate from

Table 2. Results of HI test against avian polyclonal chicken antisera to avian paramyxovirus.

Samples	HI titer against standard sera[B]				
	APMV-1	APMV-2	APMV-3	APMV-6	APMV-7
Scolopacidae/*Arenaria interpres*	—	—	—	—	—
Scolopacidae/*Arenaria interpres*	10	80	10	20	10
Scolopacidae/*Arenaria interpres*	40	80	—	—	10
Charadriidae/*Charadrius semipalmatus*	—	80	—	20	10
Scolopacidae/*Tringa melanoleuca*	10	160	10	20	10
Control[A]	160	640	320	320	320

[A]HI titer against specific standard antigen.
[B]HI titers are expressed as reciprocal of the highest dilution causing inhibition of 8 hemagglutinating units (HAU) of virus/50 μl.

49.39% to 0.20%. The low number of samples collected from birds of the family Anatidae was certainly influenced by the capture method that we used for most of the birds (mist nets). Fourteen of our samples were collected from birds from the Spheniscidae family, migratory penguins that arrive annually on the coast of the state of Rio de Janeiro in spring, August through November. We captured 494 birds; 75.1% and 24.9% of these were migratory and resident birds, respectively, and the resident birds belonged to the families Charadriidae (*Charadrius collaris*), Strigidae (*Speotyto cunicularia*), Cuculidae (*Crotophaga ani*), and Anatidae (*Dendrocygna viduata*). Since 2005, our group has monitored AIV and APMV circulation in resident and migratory birds as part of the effort to survey avian pathogens in wild birds in Brazil, following the contingency plan of the Brazilian Agriculture Ministry (6).

Data on the distribution of APMVs and AIV in shorebirds in Brazil are also limited. The circulation of influenza A H1N1 and H3N2 viruses has been observed among wild and domestic birds (3,4,20,24). In our study, all hemagglutinating samples were negative for AIV by RT-PCR; however, RT-PCR was able to characterize 1.01% (05/494) of the samples as containing APMV. As described in Table 2, 0.80% (4/494) of the total samples were found to contain APMV-2 by HI test. Of these four APMV-2 samples, two were isolated from the species *Arenaria interpres*, one was isolated from *Tringa melanoleuca*, and one was isolated from *Charadrius semipalmatus*; the samples had HI titers between 80 and 160. Although the sample from Scolopacidae/*Arenaria interpres* presented HI titer equal to 40 against serum to APMV-1, it could not be amplified with NDV-specific primers. Among birds of the species *Arenaria interpres*, the prevalence of APMV-2 was 2.1% (02/94), although one sample could not be characterized using the HI test. Electron microscopy revealed 700-nm rounded virus particles decorated with 8–10-nm projections and typical helical free "herringbone" nucleocapsids (Fig. 1). In addition, microscopy revealed filaments with a length compatible with paramyxovirus nucleoproteins (16). In accordance with previous reports on the circulation of influenza viruses in wild birds, the results varied widely among species and even within the same species of different nesting or wintering sites. The absence of virus detection in relation to the family Anatidae may be explained by the small number of birds captured (9/494).

Similarly, in a study developed among shorebirds and gulls captured in ten U.S. states and three countries in the Caribbean and South America (9), the prevalence of APMVs was 0.7% (60/9128), and only APMV-1 and APMV-2 were detected. Two isolates (APMV-2) were obtained from gulls, whereas 58 isolates (APMV-1/41 isolates and APMV-2/17 isolates) were obtained from shorebirds. All of the positive shorebirds were samples from Delaware Bay, U. S. A., and 78% of these isolates were ruddy turnstones (*Arenaria interpres*). In the materials collected from these ruddy turnstones, the total APMV-2 prevalences were 0.5% (2/394) and 1.8% (13/735) in 2001 and 2002, respectively. However, in the following year (2003), no APMV-2 was observed in these species in Delaware Bay (9). These results demonstrate the variation in APMV prevalence.

The first isolate of APMV-2 was obtained in 1956 in south California (Yucaipa, U. S. A.) from a flock of 3-wk-old chickens presenting infectious laryngotracheitis (29). Since then, these viruses have been isolated from chickens, turkeys, and feral birds across the globe; they have been more frequently isolated from passerine birds, but also commonly from psittacine birds. APMV-2 also can cause egg production losses; however, its rate of spread and the mortality have been unusually high (29). In Brazil, for the first time, our group documented the detection of APMV-2 and APMV-10 among Magellanic penguins (*Spheniscus magellanicus*) on the northeastern coast, approximately 4000 km from their breeding colonies (13). The APMV-10 isolate was previously described by Miller *et al.* (17) in rockhopper penguins from the Falkland Islands.

In our analysis, the resident species that were negative for APMVs and IAVs included *Charadrius collaris*, *Gallinago paraguaiae*, *Vanellus chilensis*, *Crotophaga ani*, *Anas bahanensis*, *Anas boschas*, *Dendrocygna viduata*, *Speotyto cunicularia*, *Butorides striatus*, *Tito alba*, *Nycticryphes semicollaris*, *Diomedea chlorinchus*, *Columbina talpacoli*, and *Chordeiles sp.* Fecal samples from other species included in our work, such as *Spheniscus magellanicus* and *Macronectes* sp., were also negative for both viruses.

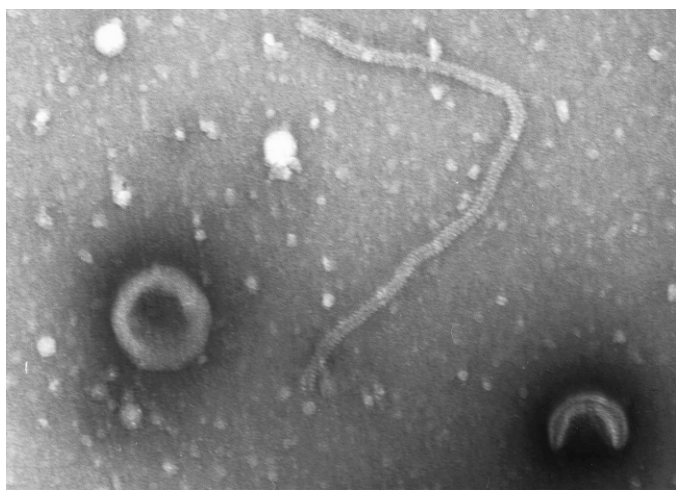

Fig. 1. An electron microscopy image of virus isolate from *Arenaria interpres*, showing enveloped pleomorphic virus and herringbone structures negatively stained with 4% phosphotungstic acid. Scale bar = 100 nm.

Regarding APMV-1 (NDV), Thomazelli *et al.* (30) were able to detect this virus in 0.7% of the samples collected by tracheal/cloacal swabs from among 1022 asymptomatic domestic and wild birds from the Brazilian coast and Amazon region using PCR. Low-virulence strains of NDV have also been regularly isolated from wild birds in the U. S. A. Migratory wild birds have been shown to transmit these viruses to free-range poultry through direct contact or by contamination of feed or water (2,15).

Although the southeast coast of Brazil contains an important complex of lakes used by migratory species to rest and feed, which allows us to monitor shorebirds (mainly Charadriiformes), the prevalence of APMVs and AIVs in wild birds (shorebirds) can be altered by factors such as site of collection, quality of samples, and species specificity (1,2,9,14,26,27). Our work collected 494 samples from 25 different species, and no AIV and NDV strains were isolated. However, four samples of Avulavirus serotype-2 (APMV-2) were found in three migratory species collected from the coast the state of Rio de Janeiro (Brazil). As Brazil is a leading producer of broiler chickens, our results reinforce the need for continuous surveillance of the potentially pathogenic viruses through the monitoring of migratory birds.

REFERENCES

1. Alexander, D. J. A review of avian influenza in different bird species. Vet. Microbiol. 74:3–13. 2000.

2. Alexander, D. J. Newcastle disease and other avian paramyxoviruses. Rev. Sci. Tech. Off. Int. Epiz. 19(2):443–462. 2000.

3. Araujo, F. A. A., R. S. T. Vianna, M. Y. Wada, E. V. Silva, L. Doretto, G. C. Cavalcante, S. M. Azevedo Jr., V. S. Magalhães, J. L. Gomes, P. V. S. Queiroz, M. E. Larrazábal, L. C. Martins, S. G. Rodrigues, and P. F. C. Vasconcelos. Inquérito sorológico em aves migratórias e residentes de Galinhos/RN para detecção do vírus da Febre do Nilo Ocidental e outros vírus. SVS Bol. Eletrônico Epidemiol. 4(2):1–12. 2004.

4. Araujo, F. A. A., R. S. T. Vianna, G. V. Andrade Filho, D. L. Melhado, B. Todeschini, G. C. Cavalcante, C. E. Fedrizzi, V. S. Magalhães, A. Scherer, M. A. B. A. Almeida, A. S. Portella, E. Santos, S. B. Scherer, L. Doretto, L. C. Martins, S. G. Rodrigues, and P. F. C. Vasconcelos. Segundo inquérito sorológico em aves migratórias e residentes do parque Nacional da Lagoa do Peixe/RS para detecção do vírus da Febre do Nilo Ocidental e outros vírus. SVS Bol. Eletrônico Epidemiol. 4(5):1–8. 2004.

5. Bengis, R. G., F. A. Leighton, J. R. Fischer, M. Artois, T. Mörner, and C. M. Tate. The role of wildlife in emerging and re-emerging zoonoses. Rev. Sci. Tech. Off. Int. Epiz. 23(2):497–511. 2004.

6. BRASIL MAPA-DAS/DAS/Programa Nacional de Sanidade Avícola. Plano de Contingência para Influenza Aviária e Doença de Newcastle. Manual de Procedimentos [Internet]. [Cited 18 September 2012]. Available from: http://www.agricultura.gov.br/arq_editor/file/Aniamal/programanacionalsanidadeavicola/panodecontingencia.pdf

7. Briand, F. X., P. Henry, P. Massin, and V. Jestin. Complete genome sequence of a novel avian paramyxovirus. J. Virol. 86(14):7710. 2012.

8. CEMAVE-IBAMA. Comitê Brasileiro de Registros Ornitológicos (2005). Listas das aves do Brasil [Internet]. [Version 22 March 2005]. Available from: http://www.ib.usp.br/cbro

9. Coffee, L. L., B. A. Hanson, M. P. Luttrell, D. E. Swayne, D. A. Senne, V. H. Goekjian, L. J. Niles, and D. E. Stallknecht. 2010. Avian paramyxoviruses in shorebirds and gulls. J. Wildl. Dis. 46(2):481–487. 2010.

10. Ellis, J. S., and M. C. Zambon. Combined PCR-heteroduplex mobility assay for detection and differentiation of influenza A viruses from different animal species. J. Clin. Microbiol. 39(11):4097–4102. 2001.

11. Ellis, T. M., R. B. Bousfield, L. A. Bissett, K. C. Dyrting, G. S. Luk, S. T. Tsim, K. Sturm-Ramirez, R. G. Webster, Y. Guan, and J. S. Malik Peiris. Investigation of outbreaks of highly pathogenic H5N1 avian influenza in waterfowl and wild birds in Hong Kong in late 2002. Avian Pathol. 33(5):492–505. 2004.

12. Fonseca, M. E. F., N. Frimer, R. E. A. Mendonça, J. N. S. S. Couceiro, and R. D. Machado. A combined staining technique developed for virus particles observation in the electron microscope. Virology 19:1. 1986.

13. Fornells, L. A., T. F. Silva, I. Bianchi, C. E. Travassos, M. H. Liberal, C. M. Andrade, M. P. Petrucci, V. F. Veiga, M. F. Vaslin, and J. N. Couceiro. Detection of paramyxoviruses in Magellanic penguins (*Spheniscus magellanicus*) on the Brazilian tropical coast. Vet. Microbiol. 156(3–4):429–433. 2012.

14. Hanson, B. A., M. P. Luttrell, V. H. Goekjian, L. Niles, D. E. Swayne, D. A. Senne, and D. E. Stallknecht. Is the occurrence of avian influenza virus in Charadriiformes species and location dependent? J. Wildl. Dis. 44(2):351–361. 2008.

15. Hines, N. L., and C. L. Miller. Avian paramyxovirus serotype-1: a review of disease distribution, clinical symptoms, and laboratory diagnostics. Vet. Med. Int. article ID 708216:1–17. doi:10.1155/2012/708216 2012.

16. Lamb, R. A., and G. D. Parks. Paramyxoviridae: the viruses and their replication. In: Fields virology, 5th ed. Knipe, D. M., and P. M. Howley, eds. Raven Press, New York. pp. 1449–1496. 2007.

17. Miller, P. J., C. L. Afonso, E. Spackman, M. A. Scott, J. C. Pedersen, D. A. Senne, J. D. Brown, C. M. Fuller, M. M. Uhart, W. B. Karesh, I. H. Brown, D. J. Alexander, and D. E. Swayne. Evidence for a new avian paramyxovirus serotype 10 detected in rockhopper penguins from the Falkland Islands. J.Virol. 84(21):11496–11504. 2010.

18. Novelli, R. Aves marinhas costeiras do Brasil: identificação e biologia. Ed. Cinco Continentes, Porto Alegre, Brazil. 1997.

19. [O.I.E.] Office Internacional des Épizooties. Manual of standards for diagnostic tests and vaccines, 7th ed. Chapter 2.3.4.; 2.3.14 [Internet]. [Accessed: 11 May 2013]. Available from: http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/ 2012.

20. Oliveira, J. G. Jr., M. S. P. Belluci, J. S. M. Vianna, C. Mazur, C. M. Andrade, L. P. L. Fedullo, C. Portz, and B. O. Loureiro. Avaliação soroepidemiológica do vírus influenza em aves domésticas e silvestres no Estado do Rio de Janeiro. Arq. Bras. Med. Vet. Zootec. 53(3):299–302. 2001.

21. Olsen, B., V. J. Munster, A. Wallensten, J. Waldenström, A. D. M. E. Osterhaus, and R. A. M. Fouchier. Global patterns of influenza A virus in wild birds. Science 312:384–387. 2006.

22. Orsi, M. A., L. Doretto Jr., S. C. A. Camillo, D. Reischak, S. A. M. Ribeiro, A. Ramazzoti, A. O. Mendonça, F. R. Spilki, M. G. Buzinaro, H. L. Ferreira, and C. W. Arns. A survey for maintenance of virulent Newcastle disease virus–free area in poultry production in Brazil. Braz. J. Microbiol. 41:368–375. 2010.

23. Palese, P., and M. L. Shaw. Orthomyxoviridae: the viruses and their replication. In: Fields virology, 5th ed. Knipe, D. M., and P. M. Howley, eds. Raven Press, New York. pp. 1647–1689. 2007.

24. Soares, P. B. M., C. Demétrio, L. Sanfilippo, A. H. N. Kawanoto, L. Brentano, and E. L. Durigon. Standardization of a duplex RT-PCR for the detection of influenza A and Newcastle disease viruses in migratory birds. J. Virol. Meth. 123(2):125–130. 2005.

25. Stallknecht, D. E. Ecology and epidemiology of avian influenza viruses in wild bird populations: waterfowl, shorebirds, pelicans, cormorants, etc. In: Proc. Fourth International Symposium on Avian Influenza. D. E. Swayne, ed. American Association of Avian Pathologists, Kennett Square, PA. pp. 61–69. 1998.

26. Stallknecht, D. E., and J. D. Brown. Wild birds and the epidemiology of avian influenza. J. Wildl. Dis. 43(3)(Supplement):S15–S20. 2007.

27. Stallknecht, D. E., and S. M. Shane. Host range of avian influenza virus in free-living birds. Vet. Res. Commun. 12:125–141. 1988.

28. Stäuber, N., K. Brechtbühl, L. Bruckner, and M. A. Hofmann. Detection of Newcastle disease virus in poultry vaccines using the polymerase chain reaction and direct sequencing of amplified cDNA. Vaccine 13(4):360–364. 1995.

29. Subbiah, M., S. Xiao, P. L. Collins, and S. K. Samal. Complete sequence of the genome of avian paramyxovirus type 2 (strain Yucaipa) and comparison with other paramyxoviruses. Virus Res. 137(1):40–48. 2008.

30. Thomazelli, L. M., J. Araujo, C. S. Ferreira, R. Hurtado, D. B. Oliveira, T. Ometto, M. Golono, L. Sanfilippo, C. Demetrio, M. L. Figueiredo, and E. L. Durigon. Molecular surveillance of the Newcastle

disease virus in domestic and wild birds on the north eastern coast and Amazon biome of Brazil. Rev. Bras. Cienc. Avic. 14(1):1–7. 2012.

31. Tong, S., S. W. W. Chern, Y. Li, M. A. Pallansch, and L. J. Anderson. Sensitive and broadly reactive reverse transcription-PCR assays to detect novel paramyxoviruses. J. Clin. Microbiol. 46(8):2652–2658. 2008.

32. Vooren, C. M., and L. F. Brusque. Avaliação das ações prioritárias para a conservação da biodiversidade da zona costeira e marinha: diagnóstico sobre aves do ambiento costeiro do Brasil, Rio Grande do Sul, Brasil [Internet]. [cited December 2004]. Available from: http://www.bdt.fat.org.br/workshop/costa/aves.

33. Warke, A., L. Appleby, and E. Mundt. Prevalence of antibodies to different avian paramyxoviruses in commercial poultry in the United States. Avian Dis. 52:694–697. 2008.

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