EVIDENCE OF ORTHO- AND PARAMYXOVIRUSES IN FAUNA FROM ANTARCTICA

Authors: Austin, F. J., and Webster, R. G.

Source: Journal of Wildlife Diseases, 29(4) : 568-571

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-29.4.568

Journal of Wildlife Diseases, 29(4), 1993, pp. 568–571

EVIDENCE OF ORTHO- AND PARAMYXOVIRUSES IN FAUNA FROM ANTARCTICA

F. J. Austin[1] and R. G. Webster[2]

[1] Health Research Council of New Zealand Virus Research Unit, University of Otago, Dunedin, New Zealand

[2] Department of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, Tennessee 38101, USA

ABSTRACT: Serum antibodies to influenza A viruses and paramyxoviruses were detected in Adelie penguin (*Pysoscelis adeliae*) and Antarctic skua (*Stercorarius skua maccormicki*) sera in the Ross Sea Dependency. An avian paramyxovirus was isolated from a penguin cloacal swab.

Key words: Antarctic, Adelie penguin, Antarctic skua, Weddell seal, influenza virus, paramyxovirus, serological survey, indirect enzyme immunoassay.

INTRODUCTION

Influenza A viruses and paramyxoviruses are widely distributed among birds in temperate regions (Alexander, 1980; Webster et al., 1992) and have been reported in Adelie penguins (*Pysoscelis adeliae*) in the eastern Antarctic (Morgan and Westbury, 1981, 1988). Wild birds are believed to play a role in the spread of the pathogenic paramyxovirus, Newcastle disease virus (Hanson, 1972), and there is compelling evidence that aquatic wild birds are the primordial reservoir of all influenza viruses for avian and mammalian species, including humans (Webster et al., 1992). Two recent seal epizootics caused by avian influenza-like viruses (Hinshaw et al., 1984) raised the question of whether seals worldwide are natural hosts of influenza viruses or whether they recently were infected from birds. Our objective was to determine the prevalence and distribution of avian influenza viruses and paramyxoviruses among Weddell seals (*Leptonychotes weddelli*), Adelie penguins and Antarctic skuas (*Stercorarius skua maccormicki*) on Ross Island in the Ross Sea Dependency, an isolated area with a limited avian and mammalian fauna.

MATERIALS AND METHODS

In November 1978 tracheal and cloacal swabs and blood samples were collected from 100 adult Adelie penguins and 60 adult Antarctic skuas near their breeding ground at Cape Bird (77°14′S, 166°28′E) on Ross Island, Antarctica. Additional tracheal and cloacal swabs were taken from 168 juvenile penguins, 85 juvenile skuas and 62 adult skuas in the same area in January 1986. Blood samples were collected only from the adult skuas. In the same month nasal swabs and blood samples were obtained from 10 juvenile and 45 adult Weddell seals on the sea-ice near Cape Armitage (77°51′S, 166°45′E). Another 182 serum samples which were collected between October 1985 and January 1986 from adult seals in the same area were kindly donated by Dr. Shelly Varca of the U.S. Antarctic Research Program. All samples were collected in the field from live-captured unanesthetized animals which were released as soon as the samples were obtained.

The samples were transported in liquid nitrogen and stored at −70 C until tested. Nasal, tracheal and cloacal swabs were tested for influenza viruses and paramyxoviruses by allantoic and amniotic inoculation into embryonated hen and duck eggs (WHO Collaborating Centers for Reference and Research on Influenza, 1982).

An indirect enzyme immunoassay (EIA) was done. Microtiter plates were coated with 200 hemagglutinating units/well of disrupted purified virus as antigen (Kida et al., 1982). Nonspecific binding sites were blocked with phosphate buffered saline (pH 7.2) (PBS) containing 1% bovine serum albumin (Calbiochem, La Jolla, California, USA), and 0.025% Tween-20 (British Drug Houses, Poole, England). Sera were diluted 1:10 and distributed into pairs of antigen-coated microtiter wells. Specific antibody binding was detected by adding either rabbit anti-goose gamma-globulin, which reacted to a titer of 1:1,000 with penguin and skua sera, or rabbit anti-seal gamma-globulin; this was followed by horse-radish peroxidase-conjugated goat anti-rabbit gamma-globulin

(Nakane and Kawaoi, 1974). The rabbit anti-goose gamma-globulin and the horse-radish peroxidase-conjugated goat anti-rabbit gamma-globulin were kindly donated by Drs. B. L. Gibbins and J. Kalmakoff, respectively, and prepared by the methods of Kawamura (1977). The rabbit anti-seal gamma-globulin was prepared by immunizing a rabbit with ammonium sulphate-precipitated Weddell seal gamma-globulin (Kawamura, 1977). After the addition of each reagent the microtiter plate was incubated for 1 hr at 37 C and then washed three times with PBS containing 0.025% Tween-20. Bound peroxidase was detected by adding orthophenylene diamine (0.04% w/v) (Sigma Chemical Company, St Louis, Missouri, USA) and hydrogen peroxide (0.3% v/v) in citrate-phosphate buffer (pH 5.0) (Diem, 1962). After 30 min at 22 C the reaction was stopped with 2 M sulphuric acid and the absorbance of the samples was read at 490 nm (A_{490}) on an EAR 400 plate reader (SLT-Labinstruments, Salzburg, Austria). The absorbance readings were corrected for non-specific binding by subtracting the absorbance of serum in uncoated wells from the absorbance of serum in antigen-coated wells. Results were recorded as the difference between the corrected absorbance of the sample and the corrected absorbance of a negative control. The cut-off value was calculated by adding three standard deviations to the mean absorbance of negative controls. Sera with absorbance values equal to or above the cut-off value were considered to contain antibody. The specificity of the assay was demonstrated by testing sera of immunized hens. Antisera against influenza A, influenza B and an avian paramyxovirus (APMV/179/78) had corrected A_{490} absorbance values of 1.167, 0.763 and 1.642, respectively, against the homologous antigens, and values ranging from 0.022 to 0.105 against the heterologous antigens.

Bird sera were tested simultaneously against influenza virus A/dk/Bav/2/77(H1N1) (A/dk/Bav) and the avian parainfluenza virus APMV/179/78. Seal sera were tested against A/dk/Bav. As a negative control, sera also were tested against an influenza B virus which infects only humans. Hyperimmune hen and seal sera were included as positive controls.

Hemagglutination-inhibition (H-I) tests were performed in microtiter plates with receptor-destroying enzyme-treated sera (WHO Collaborating Centers for Reference and Research on Influenza, 1982). Bird sera collected in 1978 were screened by H-I against 15 influenza virus hemagglutinins (avian H3–H11, human H1–H3, swine H1 and equine H3 and H7) and APMV/1, APMV/4 and APMV/179/78.

Neuraminidase-inhibition (N-I) tests (Aymard-Henry et al., 1973) were done with avian influenza virus neuraminidases N1–N5 and N7–N9 on 14 penguin sera and 12 skua sera collected in 1978. For electron microscopy, the virus was negatively stained with phosphotungstic acid by the methods of Nermut (1982) and viewed with a Siemens model 102 electron microscope.

TABLE 1. Enzyme immunoassay-binding antibodies in avian sera from Cape Bird, Antarctica, 1978 to 1986.

Year	Species	Antibody	
		A/dk/Bav	APMV/179/78
1978	Penguin	0/100[a]	17/100
	Skua	4/60	5/60
1986	Skua	7/63	1/63

[a] Number positive/number tested.

RESULTS

No influenza viruses were isolated but a paramyxovirus was grown from a cloacal swab taken from an adult penguin in November 1978. The virus (APMV/179/78) was classified as a paramyxovirus by its morphology when viewed by electron microscopy.

No influenza virus antibodies were detected in the seal sera. Adelie penguin sera did not contain EIA-binding antibodies against influenza A virus but 11 of 123 skua sera did (Table 1). The absorbance values of the positive sera ranged from 3.2 to 15.3 standard deviations greater than the negative mean. There were EIA-binding antibodies against APMV/179/78 in both penguin and skua sera with absorbance values of the positive sera ranging from 3.2 to 10.9 standard deviations above the negative mean for penguins and 3.0 to 4.4 standard deviations above the negative mean for skuas. None of the sera reacted with influenza B antigen.

The only H-I antibodies present in penguin and skua sera were directed against influenza antigen H10. Twenty-four of 91 penguin sera and four of 60 skua sera had reciprocal titers of ≥ 20. There was no apparent relationship between the sera positive by indirect EIA and those positive by H-I.

Of the limited number of sera tested for N-I antibodies one penguin serum and one skua serum inhibited influenza antigen N2 and another skua serum (which was positive by indirect EIA) inhibited influenza antigen N8.

DISCUSSION

The failure to isolate influenza viruses from seals in the Ross Sea Dependency or to detect influenza virus antibodies in their sera is evidence that seals are not normally infected with these viruses. However, the isolation of an avian paramyxovirus from an apparently healthy Adelie penguin at Cape Bird and the presence of paramyxovirus and influenza virus antibodies in penguins and skuas showed that these viruses do infect the avifauna of the Ross Sea Dependency.

The lack of correlation between the results of the H-I and indirect EIA tests may be because they detect different types of antibody and have different sensitivities (de Boer et al., 1990). Also a variable and transient response to myxovirus infections by penguins and skuas, as occurs in birds in temperate regions (Slemons and Easterday, 1974; Bahl and Pomeroy, 1977; Hinshaw et al., 1978), would cause discrepancies between the results of the two tests. Whether all influenza virus antibodies were due to infection with a single influenza subtype cannot be determined. The H10 specificity of the H-I-positive penguin and skua sera is evidence that this was the hemagglutinin of the virus with which they were infected, but virus isolation will be necessary to verify this.

The identity of APMV/179/78 with a paramyxovirus isolated from a penguin at Casey, some 4,000 km away (Morgan and Westbury, 1981; Alexander et al., 1989), the occurrence of avian paramyxovirus and influenza virus antibodies in the sera of penguins at Casey (Morgan and Westbury, 1981) and the presence of virus antibodies in skua sera collected at Cape Bird eight years apart are evidence that myxoviruses are widespread and persistent in the Antarctic. The spread of the viruses within the Antarctic continent and between Antarctic and temperate regions could be explained by skua feeding behavior and bird movements. Skuas feed on penguin eggs, chicks and dead birds and may acquire infections in this way. Penguins move between rookeries during both the breeding and the non-breeding seasons (Ainley et al., 1983; Davis and Miller, 1992) and in the austral winter, skuas and penguins travel long distances to places where contact with other birds could allow virus transmission to occur (Kinsky, 1970).

The ecology of influenza virus in the Antarctic will not be completely understood until influenza viruses have been isolated from birds there. The failure to isolate influenza viruses from adult birds in November and young birds in January could be due to timing of the sample collection; Kawaoka et al. (1988) has shown that the distribution of influenza viruses in other birds is seasonal. Future studies on the ecology of avian influenza viruses in the Antarctic will require the regular collection of samples for virus isolation throughout the breeding season. This need not involve stressing the birds by handling because it has been shown that influenza viruses and paramyxoviruses can be isolated readily from freshly voided feces (Kawaoka et al., 1988).

ACKNOWLEDGMENTS

We thank Kerry-Jayne Wilson and Tony Robinson for skills in the field and Julie Anderson, Sue Green, Jane Bruce and Cathy McCaughan for excellent technical assistance. We are grateful for support from the New Zealand Antarctic Programme and U.S. Navy VXE-6 Squadron. This work was supported in part by a grant from the National Institutes of Health AI 29680, by Cancer Center Support grant CA 21765 and by the American Lebanese Syrian Associated Charities.

LITERATURE CITED

AINLEY, D. G., R. E. LERESESCHE, AND W. J. L. SLADEN. 1983. Breeding biology of the Adelie penguin. University of California Press, Berkeley, California.

ALEXANDER, D. J. 1980. Avian paramyxoviruses. Veterinary Bulletin 50: 737–752.

———, R. J. MANVELL, M. S. COLLINS, S. J. BROCKMAN, H. A. WESTBURY, I. MORGAN, AND F. J. AUSTIN. 1989. Characterization of paramyxoviruses isolated from penguins in Antarctica and sub-Antarctica during 1976–1979. Archives of Virology 109: 135–143.

AYMARD-HENRY, M., M. T. COLEMAN, W. R. DOWDLE, W. G. LAVER, G. C. SCHILD, AND R. G. WEBSTER. 1973. Influenzavirus neuraminidase and neuraminidase-inhibition test procedures. Bulletin of the World Health Organization 48: 199–202.

BAHL, A. K., AND B. S. POMEROY. 1977. Experimental exposure of Franklins' gulls (*Larus pipixcan*) and mallards (*Anas platyrhynchos*) to a turkey influenza A virus A/turkey/Minn/BF/72 (Hav6Neq2). Journal of Wildlife Diseases 13: 420–426.

DAVIS, L. S., AND G. D. MILLER. 1992. Satellite tracking of Adelie penguins. Polar Biology 12: 503–506.

DE BOER, G. F., W. BACK, AND A. D. M. E. OSTERHAUS. 1990. An ELISA for detection of antibodies against influenza A nucleoprotein in humans and various animal species. Archives of Virology 115: 47–61.

DIEM, K. (editor). 1962. Documenta Geigy scientific tables, 6th ed. Geigy Pharmaceuticals, St. Leonards, New South Wales, Australia, pp. 314–315.

HANSON, R. P. 1972. Newcastle disease. *In* Diseases of poultry, 6th ed., M. S. Hofstad, B. W. Calnek, C. F. Helmboldt, W. M. Reid, and H. W. Yoder (eds.). Iowa State University Press, Ames, Iowa, pp. 619–656.

HINSHAW, V. S., R. G. WEBSTER, AND B. TURNER. 1978. Novel influenza A viruses isolated from Canadian feral ducks: Including strains antigenically related to swine influenza (Hsw1N1) viruses. Journal of General Virology 41: 115–127.

———, W. J. BEAN, R. G. WEBSTER, J. E. REHG, P. FIORELLI, G. EARLY, J. R. GERACI, AND D. J. ST. AUBIN. 1984. Are seals frequently infected with avian influenza viruses? Journal of Virology 51: 863–865.

KAWAOKA, Y., T. M. CHAMBERS, W. L. SLADEN, AND R. G. WEBSTER. 1988. Is the gene pool of influenza viruses in shorebirds and gulls different from that in wild ducks? Virology 163: 247–250.

KAWAMURA, A. 1977. Fluorescent antibody techniques and their applications, 2nd ed. University of Tokyo Press, Tokyo, Japan, pp. 13–37.

KIDA, H., L. E. BROWN, AND R. G. WEBSTER. 1982. Biological activity of monoclonal antibodies to operationally defined antigenic regions on the hemagglutinin molecule of A/Seal/Massachusetts/1/80 (H7N7) influenza virus. Virology 122: 38–47.

KINSKY, F. C. 1970. Annotated checklist of the birds of New Zealand including the birds of the Ross Dependency. A. H. and A. W. Reed (eds.). Wellington, New Zealand, pp. 16, 52.

MORGAN, I. R., AND H. A. WESTBURY. 1981. Virological studies of Adelie penguins (*Pygoscelis adeliae*) in Antarctica. Avian Diseases 25: 1019–1026.

———, AND ———. 1988. Studies of viruses in penguins in the Vestfold Hills. Hydrobiologia 165: 263–269.

NAKANE, P. K., AND A. KAWAOI. 1974. Peroxidase-labeled antibody. A new method of conjugation. Journal of Histochemistry and Cytochemistry 22: 1084–1091.

NERMUT, M. V. 1982. Advanced methods in electron microscopy. *In* New developments in practical virology, C. R. Howard (ed.). Alan R. Liss, New York, New York, pp. 1–58.

SLEMONS, R. D., AND B. C. EASTERDAY. 1974. Host response differences amongst 5 avian species to an influenza virus—A/turkey/Ontario/7732/66(Hav2N?). Bulletin of the World Health Organization 47: 521–525.

WEBSTER, R. G., W. J. BEAN, O. T. GORMAN, T. M. CHAMBERS, AND Y. KAWAOKA. 1992. Evolution and ecology of influenza A viruses. Microbiological Reviews 56: 152–179.

WHO COLLABORATING CENTERS FOR REFERENCE AND RESEARCH ON INFLUENZA. 1982. Concepts and procedures for laboratory-based influenza surveillance. U.S. Public Health Services, Centers for Disease Control, Atlanta, Georgia, pp. B7–B12, B17–B35.

Received for publication 17 February 1993.