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Experimental infection of Common Terns with Tern virus: Influenza Virus A/Tern/South Africa/1961

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In 1961 an epizootic occurred among Common Terns along the coastline of the Cape Province, South Africa, and the causative virus was isolated and classified as Influenza A/Tern/South Africa/1961 (Becker, 1966). The pathogenesis of experimental Tern virus infection has been described in chickens (Becker & Uys, 1967; Uys & Becker, 1967), but a method of capturing live, healthy terns was eventually devised and fifteen Common and two Swift Terns were obtained for experimental purposes. The aims with the Common Terns were to confirm that the disease seen during the epizootic could be reproduced experimentally, to study the distribution of virus in the tissues of infected birds, and to determine possible means of spread of infection in the field. The Swift Terns were obtained with a view to confirming earlier observations that they suffered no ill-effects from the injection of Tern virus.

MATERIALS AND METHODS

Virus strains

The prototype strain of Tern virus (Becker, 1966) was used at the first or second allantoic passage in embryonated eggs.

Chickens and eggs

Leghorn-Australorpe-cross chickens and eggs were used.

Terns

The Common Terns (*Sterna hirundo*) and the Swift Tern (*Sterna bergii*), like the chickens, were housed in 24 in. × 18 in. × 12 in. cages. The terns were kept healthy and adequately nourished, but the Common Terns required force-feeding; the diet consisted only of sea-fish given two or three times per day.

Autopsy, virus titration, haemagglutination-inhibition (HI) tests

The details of these procedures have been described previously (Becker, 1966).

Immunofluorescence

Immunofluorescent studies were performed on tissues which were removed at autopsy, fixed in cold ethanol and embedded in low-melting-point paraffin wax according to the method of Sainte-Marie (1962). Sections were stained for viral

Table 1. *Virological data on experimentally infected Common Terns*

Tern no.	Inoculation with Tern virus: EID_{50}*	route	Result	Route of re-inoculation with 10^4 EID_{50} of Tern virus	Result	Post mortem virus titrations: Blood	Muscle	Liver	Lung	Heart	Kidney	Brain	Cloacal swab	Palatal swab
1	10^4	CN	Died within 3 days	—	—	6·9†	6·5	5·3	6·5	5·5	6·7	3·3	+	+
2	10^4	IM				5·7	4·7	5·3	6·5	5·9	6·9	3·5	NE	NE
3	10^4	IM				1·7	3·7	3·9	5·5	5·9	ND	3·9	3·0	3·0
4	10^3	CN	Unaffected	IM	Died < 3 days	NE	> 4·5	5·9	5·9	6·9	5·9	4·3	3·5	3·0
5	10^2	CN		IM	Died < 3 days	6·3	4·7	4·1	6·5	5·1	6·5	2·9	> 3·5	4·1
6	10	CN		CN	Died < 4 days	4·3	4·7	4·3	6·0	4·9	6·7	4·3	4·1	4·1
7	10	CN		CN	Recovered. Autopsied on 14th day. HI antibody titre > 40	NE	—	—	—	—	—	—	—	—
8	1	CN		IM	Died < 2 days	5·5	4·9	4·5	5·9	4·5	4·1	3·1	NE	—
9	1	CN		CN	Died < 7 days	3·9	4·1	—	5·9	3·9	5·1	4·1	—	3·3

* Egg infective $doses_{50}$.

† Log_{10} EID_{50} per g.

CN, Conjunctival/intranasal; +, virus present; NE, not examined; IM, intramuscular; —, no virus present.

antigen by the indirect method using Tern virus strain-specific antiserum prepared in guinea-pigs, followed by a rabbit anti-guinea-pig gamma globulin labelled with fluorescein-isothiocyanate (Antibodies Incorporated, Davis, California).

RESULTS

Controls

Three Common Terns and one Swift Tern were used as uninfected controls and autopsied on receipt at the laboratory; no abnormalities were detected and no histological or virological evidence of infection with Tern virus was obtained. A fourth Common Tern died on the fifteenth day of captivity owing to difficulty with feeding, and a fifth died from pulmonary aspergillosis (*Aspergillus fumigatus*) after 3 weeks in captivity; these also showed no evidence of Tern virus infection.

Experiment 1

Approximately 10^4 egg infective doses $(EID)_{50}$ of freshly passed Tern virus were administered to one Common Tern by the conjunctival/intranasal (CN) route; half the inoculum was introduced into the right conjunctival sac and half into the right nostril. Two Common Terns were injected intramuscularly (IM) with the same amount of virus. All three birds died within 3 days of infection during an illness which showed the following features: the birds became apathetic and their feathers were ruffled; then their heads and wings drooped and they preferred to close their eyes; soon they were no longer able to remain standing but gradually sagged to the ground and finally collapsed and died (Plate 1). Virus was found in all the specimens taken at autopsy including blood, breast muscle, liver, lung, heart, kidney, brain, cloacal and palatal swabs (Birds 1, 2, 3, Table 1). The droppings of one bird which was injected IM were examined twice daily for Tern virus, which was detected on the day preceding death. No HI antibodies to Tern virus were detected in pre-inoculation serum samples tested at an initial dilution of 1/5. Control 3-day-old chickens received the above dose of Tern virus CN or IM and all died showing the features of Tern virus infection previously described (Becker & Uys, 1967).

Experiment 2

Six Common Terns (birds 4–9, Table 1) received from 1 to 10^3 EID_{50} of Tern virus by the CN route, but the terns were unaffected by these doses of virus. No HI antibodies were found in pre-inoculation serum diluted 1/5, and 9 days after inoculation titres of 5 were found only in birds 7 and 9. The Swift Tern was injected IM with 10^3 EID_{50} of Tern virus and had developed an HI antibody titre of 2560 nine days later without ill-effect. Half of the control 3-day-old chickens died after inoculation CN with 10^3 EID_{50} of Tern virus.

Experiment 3

The six Common Terns from the previous experiment were re-inoculated after an interval of 19 days with 10^4 EID_{50} of Tern virus using the CN or IM route (birds 4–9, Table 1). The three terns injected IM died within 3 days; one inoculated

by the CN route died within 5 days, another within 7 days, and the third became ill but recovered and had a serum HI antibody titre of over 40 when it was autopsied on the 14th day. Virus was detected in the autopsy specimens from all except the last-mentioned bird. The Swift Tern was re-injected using 10^4 EID_{50} of Tern virus: it showed no ill effect, maintained its HI antibody titre and no virus was isolated from its tissues at autopsy 2 weeks later. Four 3-week-old chickens inoculated CN and five 3-day-old chickens injected IM served as controls; all died within 7 days.

Experiment 4

Lice (*Austromenopon* species) were transferred daily from the Common Terns used in Exp. 3 to another Common Tern which remained well during the observation period of 7 weeks: no virus was isolated from its tissues. No virus was cultured from eight lice taken from Tern 4 (Table 1) at the time of autopsy. The diet of these lice consists principally of dead feather material.

Immunofluorescence

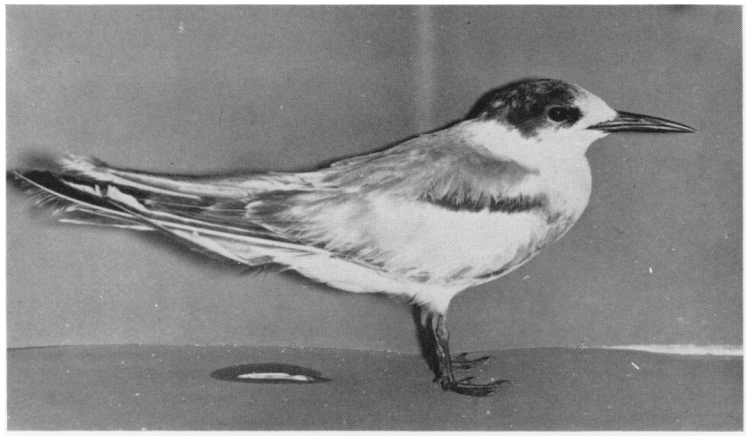
Immunofluorescent studies were carried out on all the Common Terns. Tern virus replication was only demonstrable in the tissues of the eight fatally infected terns. The sites of virus replication were in the lung in all eight birds, in the heart, skeletal muscle and brain in seven, in the kidney in three, in the spleen in all five cases in which it was examined, and in the cloacal and palatal glands in two of six birds. No specific fluorescence was detected in the liver, and the blood clot and blood smears of two terns were also examined with negative results.

CONCLUSIONS

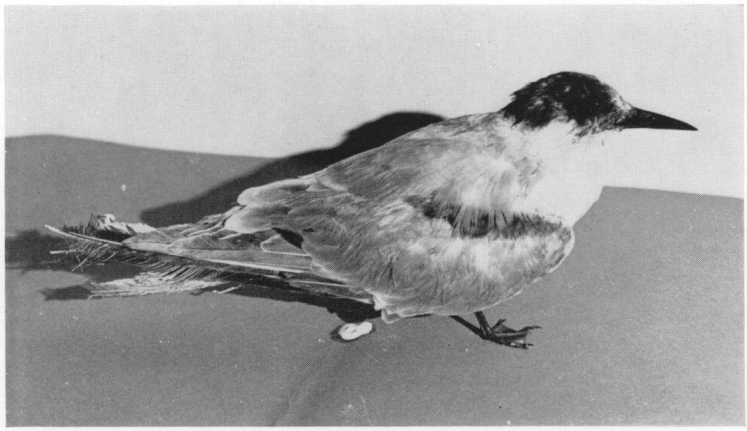
The acute illness with high mortality which affected Common Terns in the 1961 epizootic was reproduced experimentally in the original host species. The same results were obtained by either CN or IM routes of infection, but a dose of 10^4 EID_{50} was necessary to produce fatal infection by CN inoculation. At death the Common Terns had a viraemia and consequently virus was detected in all the tissues; however, immunofluorescent studies showed that the sites of virus replication were usually in muscle, heart, brain, lung, spleen, and sometimes kidney, but not in the liver. Virus was usually isolated from the palatal and cloacal swabs of the birds and viral antigen was demonstrated in the glands of the palate and cloacal mucous membrane in some birds by means of specific immunofluorescence. It was confirmed that a single dose of Tern virus injected IM had no apparent ill effect on the Swift Tern, but produced high titre circulating antibodies.

DISCUSSION

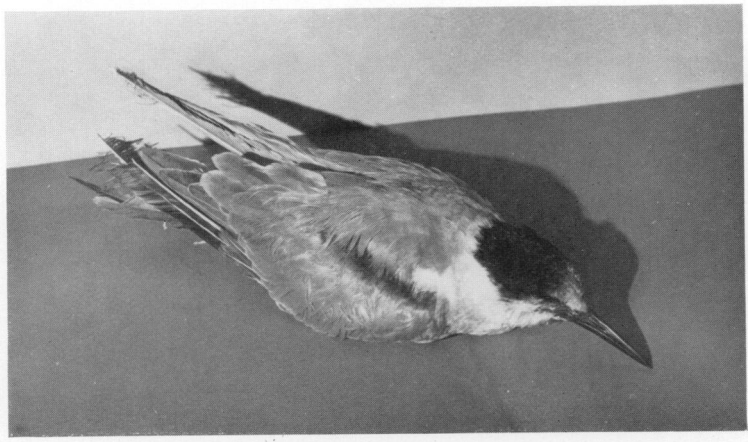
Cloacal and palatal secretions or excretions seem the likely source of virus for spread of infection, which might occur from contact with excreta, or as a result of fighting or sexual activity. In the 1961 epizootic the preceding conditions of stress might have converted a latent into an overt infection and precipitated the outbreak by lowering the threshold of resistance and thus favouring the spread



(*a*)



(*b*)



(*c*)

(*Facing p.* 65)

of the infection. The role of blood-sucking arthropods in the spread of this infection is unknown. Sea-birds may be a source of infection of domestic poultry with myxoviruses, as discussed in a previous paper (Becker, 1966). The outbreak in chickens in Scotland in 1959 (Dr J. E. Wilson, personal communication) and the Tern epizootic in 1961 were caused by influenza A viruses with closely related strain specific antigens which were unrelated to those of any previously known influenza A viruses. Recently strains of influenza A related to the Tern and Scottish viruses were isolated from turkeys in Canada (Dr G. Lang, personal communication). This lends further support to the hypothesis that migrating sea-birds such as the Common Tern may transmit avian influenza A viruses to domestic poultry.

SUMMARY

Experimental infection of captive Common Terns with Influenza virus A/Tern/South Africa/1961 reproduced the disease seen in the 1961 epizootic during which Tern virus was originally isolated. Infected terns excreted virus in their droppings. At death a viraemia was present but immunofluorescent studies determined the sites at which virus reproduction occurred. A Swift Tern suffered no ill effect from the injection of Tern virus but developed HI antibodies. The role of migrant sea-birds in spreading avian influenza is briefly discussed.

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EXPLANATION OF PLATE

Common Terns, approximately one quarter life size.

(*a*) Healthy Common Tern (No. 2, Table 1). Photograph taken before inoculation with Tern virus.

(*b*) and (*c*) Sick Common Tern (no. 3, Table 1) photographed in the late stages of infection at 68 and 70 hr. respectively after inoculation with Tern virus. The bird died at 71 hr.