A POXVIRUS ISOLATED FROM SILVEREYES (Zosterops lateralis) FROM LOWER HUTT, NEW ZEALAND 1

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A POXVIRUS ISOLATED FROM SILVEREYES (*Zosterops lateralis*) FROM LOWER HUTT, NEW ZEALAND[1]

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Abstract: Poxviruses were isolated from skin lesions of eight silvereyes during 1969-71. In all cases, characteristic virus particles were seen in extracts of the lesions and all isolates produced pocks on the chorioallantoic membrane of embryonated chicken eggs. Comparison by immunodiffusion showed that the viruses were antigenically identical, and that they differed from poxviruses recently isolated from domestic fowls, pigeons and a canary.

INTRODUCTION

Poxviruses have been isolated from a wide range of birds in many parts of the world.[3] The first report of pox-like lesions in a wild bird in New Zealand was that of Westerskov[8] who found them in a pipit *(Anthus novaeseelandiae)* but, as with later reports of infected pipits,[6] diagnosis was based merely on the macroscopic appearance of the lesions. In 1965, one of us (PCB) found pox-like lesions on the feet of three of 95 silvereyes trapped in Lower Hutt. These birds, caught in July and September were submitted to the Department of Agriculture's Diagnostic Station at Wallaceville who reported (in litt. 22nd October, 1965): "typical pox lesions seen; pox type virus isolated".

Despite the banding of 957 silvereyes in Lower Hutt during 1966 and 1967, no further pox lesions were noticed until 1968 when five were seen in May, one in June, one in July and two in September. The last three of these birds, plus five others collected during the winters of 1969-71, were forwarded to the Virus Research Unit, University of Otago. Infected birds came from gardens in residential areas of the Hutt Valley and also from native forest in the nearby Orongorongo Valley.

This article describes the isolation of a poxvirus from these silvereyes and the result of immunodiffusion tests in which it was compared with poxviruses isolated from fowls, pigeons and a canary.

MATERIALS AND METHODS

Scabs and tissues from the lesions were extracted in 50% glycerol in distilled water by grinding with sand in a mortar. The crude extracts were diluted with distilled water, and clarified by slow speed centrifugation (1,000 rpm for 10 minutes). The virus was deposited by centrifuging at 15,000 rpm for 20 minutes and a small sample was stained with phosphotungstic acid for examination by electron microscopy.

The remainder of the deposit was suspended in 1% bovine albumin in Hanks' balanced salt solution and inoculated onto the chorioallantoic membranes (CAM) of 12-day embryonated hen eggs which were then incubated at 35 C for 4 days before the CAMs were removed and examined for pocks.

Immunodiffusion antigens were prepared from infected CAMs by shaking them with glass beads in sterile buffer and centrifuging at 2,000 rpm for 10 minutes.

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The supernatant fluid was the antigen.

Antisera were prepared in domestic fowls by inoculating virus into the feather follicles and onto a scarified area of the skin causing an active infection. Fourteen days later the fowls were given an intramuscular injection of equal parts of virus antigen and Freund's complete adjuvant. They were given further intramuscular injections of antigen at weekly intervals and were bled out on the 31st day.

Immunodiffusion experiments were set up in patterns of wells cut in a gel composed of 1% ionagar No. 2 in 8% sodium chloride and preserved with 0.05% sodium azide.[1,5] Tests for specific inhibition of precipitation were performed by pretreating serum wells with antigens before setting up the immunodiffusion tests.[2]

RESULTS

Seven of the eight silvereyes which were examined in the laboratory had only a single lesion which was located on the foot or lower leg on four birds, and on the vent, mandible or side of the head on the other three birds. The eighth bird had two lesions on the foot and leg. The lesions ranged in severity from mild erythema and slight swelling to severe swelling with ulceration and an overlying scab (Fig. 1).

Poxviruses were isolated from all eight birds. In each case typical poxvirus particles were seen by electron microscopic examination of an extract of the lesion. The particles were roughly eliptical with dimensions 275-300 nm by 250-275 nm. The surface was covered with irregularly arranged filaments giving an appearance identical with that of other avian poxviruses, and quite distinct from the regular pattern of filaments seen on the surface of paravaccinia viruses.

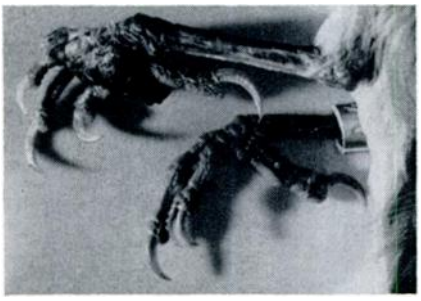

FIGURE 1. A poxvirus lesion on the foot of **Zosterops lateralis.**

The viruses multiplied on the CAM of embryonated hen eggs and produced pocks. After 96 hours incubation the pocks were observed as round, flat, creamy-white lesions between 1 and 2 mm in diameter. Characteristic cytoplasmic inclusion bodies were seen in sections of infected CAMs.

The only silvereye isolate tested was avirulent for domestic fowls. The development of specific antibodies indicated that infection occurred when the virus was injected into feather follicles and onto scarified skin but no lesions developed. By contrast a poxvirus recently isolated from domestic fowls caused erythema and scabbing which were first discerned on the 3rd day after the virus was injected and persisted until the scabs sloughed off on the 15th day. Secondary lesions developed about the head.

The immunodiffusion line patterns produced by nine extracts of poxvirus-infected CAMs reacting against the serum of a fowl hyperimmunized against silvereye poxvirus have been examined. The line patterns formed by six different silvereye isolates contained three components. Two of the precipitin lines were rather faint and diffuse and difficult to photograph, but the third one was sharp and joined between adjacent extracts indicating that it was formed by an identical antigen in all six extracts. The patterns formed by poxviruses recently isolated from domestic fowls, pigeons and a canary contained two or three components, but they all lacked the antigen responsible for the sharp precipitin line (Fig. 2). The uniqueness of this antigen for silvereye poxvirus was confirmed by pretreating the antiserum wells with poxvirus extracts. The line was completely inhibited by pretreatment with an extract of silvereye poxvirus, but was unaffected by extracts of poxviruses from the other three species, or by uninfected CAM (Fig. 3).

Similar immunodiffusion tests with antisera prepared against the poxvirus isolates from fowls and pigeons indicated that these two agents and that from a canary differed from each other.

FIGURE 2. Immunodiffusion reactions. Poxvirus antigens:
wells 1, 3 and 5, from silvereyes;
well 2, from pigeons;
well 4, from a canary;
well 6, from fowls.
Antibody: well 7, antiserum prepared against a silvereye poxvirus isolate.

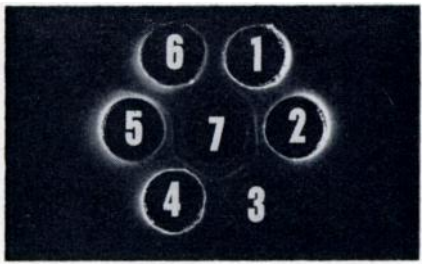

FIGURE 3. Specific inhibition of precipitation. Pretreatment: Poxvirus antigens from fowls (well 1); pigeons (well 2); canary (well 4); silvereyes (well 6). Uninfected CAM extract (well 5); saline (wells 3 and 7).
Immunodiffusion: Well 7, poxvirus antigen from silvereyes; wells 1, 2, 3, 4, 5 and 6, antiserum prepared against a silvereye poxvirus isolate.

DISCUSSION

The agent isolated from silvereyes had the typical morphology of a poxvirus. Although it was not possible to compare it with reference strains of fowl poxvirus and pigeon poxvirus, the results of the immunodiffusion tests indicate that it is antigenically different from poxviruses recently isolated from fowls, pigeons and a canary. It induced gross skin lesions on some silvereyes, but appeared to cause them little distress, and it failed to cause clinical lesions or other signs of disease in four domestic fowls which were infected with live virus in the course of immunization.

Even allowing for some infections being overlooked, the incidence of severe lesions (Fig. 1) was probably well under 1% of the 8748 silvereyes handled during 1960-1971. All the infected birds were taken in winter or spring when silvereyes are trapped in large numbers for banding. The wire-netting traps occasionally caused minor abrasions to the birds and, in this way, might conceivably facilitate the spread of the virus. So few silvereyes visit the traps in summer that any infected birds present at that season would probably remain undetected.

The isolation of this virus raises the possibility that silvereyes, which immigrated to New Zealand about 1856,[4] may have brought with them a poxvirus which could have played some part in the rapid decline of several native birds during the latter half of the nineteenth century as poxviruses seem to have done in Hawaii.[7] Hopefully, future work will show whether the virus here reported is the same as that occurring in pipits and whether it occurs also in silvereyes in Eastern Australia or Tasmania whence the New Zealand population originated.

LITERATURE CITED

1. BENEDICT, A. A., R. T. HERSH, and C. LARSEN. 1963. The temporal synthesis of chicken antibodies. The effect of salt on the precipitin reaction. J. Immunol. 91: 795-802.

2. BJORKLUND, B. 1952. Specific inhibition of precipitation as an aid to antigenic analysis with gel diffusion method. Proc. Soc. exptl. Biol. Med. 79: 319-324.
3. KIRMSE, P. 1967. Pox in wild birds — an annotated bibliography. Wildl. Dis. No. 49. 10 pp.
4. OLIVER, W. R. B. 1955. *New Zealand Birds.* A. H. & A. W. Reed, Wellington, 661 pp.
5. OUCHTERLONY, O. 1958. Diffusion-in-gel methods for immunology analysis. Progr. Allergy 5: 1-78.
6. QUINN, J. P. 1971. Suspected case of bird pox in a small population of New Zealand pipits. Notornis 18: 217.
7. WARNER, R. E. 1968. The role of introduced diseases in the extinction of the endemic Hawaiian avifauna. Condor 70: 101-120.
8. WESTERSKOV, K. 1953. Bird pox in a New Zealand pipit. Notornis 5: 168-170.

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