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Immunization Against Psittacine Pox

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SUMMARY

Pox virus isolated from psittacine birds was used as a vaccine in trials with love birds (*Agapornis roseicollis*). The vaccine was applied by wing-web puncture using single- and double-needle applicators. Immunity was effective against challenge with virulent psittacine pox virus administered via the feather follicle/thigh. When unvaccinated contact control birds were placed with the vaccinated individuals immediately post-vaccination, virus spread was evident. However, susceptible birds placed with vaccinated ones at 27 days postvaccination remained uninfected for 11 weeks. The importance of a high vaccine virus titer was observed.

RESUMEN

Se utilizó virus de viruela aislado de psitácidos como vacuna en experimentos con periquitos (*Agapornis roseicollis*). La vacuna fué administrada por punción en el ala usando aplicadores de una o dos agujas. La inmunidad adquirida fué efectiva contra un desafío hecho con virus virulento de viruela de psitácidos administrado removiendo plumas del muslo y aplicando el virus. El virus se difundió a controles no vacunados puestos en contacto con las aves vacunadas inmediatamente después de la vacunación. Sin embargo, aves susceptibles colocadas en contacto con aves vacunadas a los 27 días después de la vacunación permanecieron sin infectarse durante 11 semanas. Se observó la importancia de un título alto del virus vacunal.

INTRODUCTION

Pox virus infection of psittacine birds has resulted in serious losses in recent years (1,2,5). The disease is seen most commonly in imported birds (1). Although commercial pox vaccines are available to immunize domestic fowl against pox, none are available for psittacines. Psittacine pox virus isolates that have been isolated and studied do not appear to be related immunologically to those affecting fowl (5). The need for immunization of psittacines with a vaccine derived from psittacine pox virus initiated this study. The results are reported herein.

MATERIALS AND METHODS

Viruses. The three isolates of psittacine pox virus used were isolated from imported birds. Some characteristics of infection and pathogenicity of isolate A have been reported by Boosinger *et al.* (1). The C isolate, obtained from pox lesions of imported psittacine birds originating in Argentina, was supplied by Dr. Jack Hanley, Poultry Diagnostic Laboratory, Dade City, Florida. Isolate C was reported to be of low virulence compared with A and B viruses and where pox epornitics were observed and assessed clinically. All three isolates were related immunologically (5).

Virus propagation. The pox viruses were propagated in 10-day-old specific-pathogen-free embryos (SPAFAS, Inc., Norwich, Conn.) according to previously described procedures (3). Five or 6 days after inoculation, the chorioallantoic membranes were harvested, triturated in nutrient broth, and tested for sterility. Before use, each virus pool was titrated in embryos. Whether used as a vaccine or for challenge, each virus possessed at least 10^4 median embryo infectious doses (EID_{50}) per ml and met minimum infectivity requirements determined with fowl and pigeon pox viruses in chickens (4). In one trial in this study, varied dosages were given to different groups of birds to determine necessary dosage requirements of each vaccine.

Psittacine birds. Peach-faced love birds (*Agapornis roseicollis*), utilized in all of the immunization trials, were under 10 weeks of age at the time of vaccination. All were hatched and reared in an aviary and appeared clinically healthy. The birds were maintained in conventional cages in isolation units.

Vaccination and challenge. The birds were vaccinated with virus via the wing web using a single- or double-needle applicator. Challenge of immunity was done by pulling the feathers from an area (about 0.5 cm) on the right thigh, lightly scarifying the denuded follicles, and then applying pox-containing nutrient broth with a cotton swab on an applicator stick. Seven days postvaccination and 7 days postchallenge, all inoculation sites were examined for lesions, or takes, typical of pox reactions (1,3,4,5).

Experimental procedure. Table 1 shows experimental design of trials 1-3. In trial 1, three groups of five birds each were vaccinated by wing stab with a commercial pox double-needle applicator. Each group received a different virus isolate. Three weeks postvaccination, virus isolate A was

Table 1. Trials 1-3. Response of love birds to vaccination with three isolates of psittacine pox virus. Vaccine administered by wing stab.[A]

Trial	Psittacine pox virus applied	Wing-web reaction from stab vaccination	Thigh reaction from follicle challenge	Conjunctival reaction in postvaccination and postchallenge period
1	A	2/5[B]	0/5	2/5
	B	4/5	0/5	1/5
	C	4/5	0/5	0/5
	None	0/5	5/5	0/5
2	C	9/12	0/12	0/12
	Contact controls	--	1/6	2/6
	Separate controls	--	4/5	0/5
3	C	15/15	0/13	0/13
	Contact controls	--	5/5	0/5
	Separate controls	--	7/8	0/8

[A]Double-needle applicator used in trials 1 and 2; single needle used in trial 3.

[B]Number of birds with pox lesions/number given virus.

given as challenge. The birds were observed postvaccination and postchallenge for conjunctival pox lesions characteristic of natural psittacine pox epornitics (1).

In trial 2, 12 birds were vaccinated with pox virus C. Six unvaccinated birds were then placed immediately with the vaccinated birds to serve as contact controls. Five unvaccinated birds were retained as an unvaccinated, but separate, control group. All birds were challenged with virus A 6 weeks later. As in the previous trial, the birds were observed for typical pox lesions.

In trial 3, 15 birds were vaccinated with virus isolate C using a single-needle applicator. Twenty-seven days after vaccination, five susceptible control birds were placed with the vaccinated birds to detect possible virus shed. All birds were challenged 54 days postvaccination.

Trial 4 (Table 2) was conducted with tenfold dilutions of pox virus C. Because all vaccine and challenge viruses in the previous trials possessed at least 10^4 EID_{50} per ml, it was desirable to determine minimum dosage requirements for immunization purposes. Each dilution of the vaccine virus was given to four birds. Six unvaccinated, unchallenged individuals were also maintained separately. Thirty-one days postvaccination, all birds were challenged.

RESULTS

Trial 1. Table 1 gives the results of vaccination with the pox virus isolates using a double-needle applicator. All birds vaccinated with virus A were effectively immunized, and less than one-half demonstrated takes from vaccination. However, in the postvaccination period but before challenge, two birds had typical conjuctival

pox lesions suggesting spread of the virus. Vaccines B and C resulted in 80 percent takes and satisfactory protection from challenge. Conjunctival pox lesions were noted in one bird given vaccine B. Since vaccine C effected the most desirable characteristics in this experiment, it was used in subsequent trials.

Trial 2. Table 1 shows the results of vaccinating birds with vaccine C. Take reactions were excellent, and the protection from challenge was complete. However, the vaccine virus was apparently transmitted to susceptible contact controls.

Trial 3. In trial 3 (Table 1), a larger number of birds were given vaccine C via the wing-web with a single-needle applicator. All birds had a postvaccination reaction at the site of inoculation, and all birds were immune from challenge. The contact controls did not become infected, as indicated by their susceptibility to the challenge virus; no conjunctival pox lesions were seen during the course of the trial. Two vaccinated birds and one unvaccinated control died from cannibalism or picking.

Trial 4. In trial 4 (Table 2), vaccine C was titrated in birds to determine an effective dosage level to be administered with a single-needle applicator. When given a $\log_{10}$ 3.62 virus titer, all birds had typical wing-web takes. All were protected from challenge. At $\log_{10}$ 2.62, protection was complete; one bird did not show a take. As noted, protection was considerably less at lower dosages.

DISCUSSION

When applied by wing stab, psittacine pox virus was capable of stimulating protection against severe pox virus challenge via the follicle-thigh. Pox virus C was used after the first trial, since it

Table 2. Trial 4. Response of love birds to vaccination with different concentrations of psittacine pox virus administered with a single-needle applicator via the wing web.

Dilution of vaccine virus	Virus concentration ($\log_{10}$ per ml)	Take reaction from vaccination[A]	Pox lesions from challenge[B]	Percent immune
10^{-1}	$10^{3.62}$	4/4	0/4	100
10^{-2}	$10^{2.26}$	3/4	0/4	100
10^{-3}	$10^{1.62}$	0/4	1/4	25
10^{-4}	$10^{0.62}$	0/4	4/4	0
10^{-5}	$10^{0.06}$	0/4	4/4	0
Unvaccinated unchallenged controls (6 birds)	--	--	--	--

[A] Number of birds with wing-web take/number vaccinated.

[B] Number of birds with pox lesion on thigh/number challenged.

appeared less pathogenic and was less likely to spread from the vaccination site. However, when contact controls were placed immediately with vaccinated birds, the virus did spread (trial 2, Table 1). In contrast, virus did not shed when susceptible controls were placed with vaccinated birds at 27 days postvaccination (trial 3, Table 1). As recommended routinely with pox vaccination of chickens and turkeys, it appears desirable to vaccinate all psittacine birds to minimize spreading potential.

At least in small psittacines, vaccination with a single-needle applicator appears preferable to vaccination with a double needle. The immune response was as satisfactory as when a double needle was employed. Moreover, trauma may be minimized. Having a pox vaccine with a high titer, preferably 10^4 or greater, was important, which confirmed the work of Winterfield and Hitchner (4). The data from these experiments are sufficiently encouraging for further trials under field conditions with larger numbers of birds. Of course, the variables in working with diverse populations of imported psittacines are great. Intercurrent virus infections may be encountered during the immunization process and must be considered in determining the efficacy and safety of vaccine application.

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