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IMMUNIZING CANADA GEESE AGAINST AVIAN CHOLERA

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Vaccination as a means of disease control has been practiced for nearly a century in both human and domestic animal populations. Recently, wild vertebrates such as mountain sheep (*Ovis canadensis*) have been vaccinated against blue tongue and contagious ecthyma (R. Lange, pers. commun.), free-living carnivores against rabies (Winkler et al. 1975), and African wildlife against rinderpest (Harthoorn and Lock 1960). However, the idea of vaccinations to control avian cholera in waterfowl, especially migratory waterfowl, is unique.

This study determined the feasibility of developing an effective, practical avian cholera

Fig. 1. Typical 1-day collection of waterfowl carcasses during an avian cholera die-off. Photo by S. Kerr.

immunization program for endangered Aleutian Canada geese (*Branta canadensis leucopareia*). These birds have a limited geographic range, nesting in the Aleutian Islands and wintering in the Central Valley of California where avian cholera has remained endemic since 1948 (Rosen and Bischoff 1949, Friend 1981). Thousands of waterfowl die annually on wintering grounds shared by the Aleutian Canada geese (Titche 1979) (Fig. 1).

A recovery plan was initiated in 1974 by the U.S. Fish and Wildlife Service, the California Department of Fish and Game, and the Alaska Department of Fish and Game to reestablish the Aleutian Canada goose. At the present time, this program involves transplanting young wild goslings from Buldir Island in the Aleutians to other selected predator-free islands in the chain during the summer breeding season (Springer et al. 1977). The geese are trapped in California for banding in the winter. This management effort lends itself to an avian cholera vaccination program. A satisfactory vaccine would protect geese when they are at greatest risk of exposure to the disease.

METHODS

Culture Selection and Characterization

A Type 1 *Pasteurella multocida* culture was isolated from the carcass of a lesser snow goose (*Chen caerulescens*) that died of avian cholera in the Central Valley of California in November 1980. The culture was inoculated into Canada geese (*Branta canadensis*)

Table 1. Summary of experimental design; number and origin of waterfowl and treatment.

Experiment no.	Waterfowl species	No. birds	Waterfowl source	Treatment
Pre-test	Canada geese	2	WI-wild	Virulence test of B-2
1	Giant Canada geese	10	NE-captive	Titration of B-2 IM route
1	Giant Canada geese	10	NE-captive	Titration of B-2 SQ route
2	Giant Canada geese	12	NE-captive	Vaccinates/challenge (Table 2)
2	Giant Canada geese	12	NE-captive	Controls/challenge (Table 2)
2	Mallards	18	MN-domestic[a]	Susceptibility to B-2
2	American coots	4	WI-wild	Susceptibility to B-2
3	Giant Canada geese	13	WI-wild	Vaccinates/challenge (Table 3)
3	Giant Canada geese	12	WI-wild	Controls/challenge (Table 3)
4	Giant Canada geese	171	NE-captive	Field Trial

[a] Pine Cone Farms, Route #3, Park Rapids, Minn. 56470.

to determine the virulence of the culture. *P. multocida* were reisolated from the liver and spleen of a goose and identified biochemically and serologically. The agar-gel diffusion test was used with reference sera obtained from U.S.D.A., National Animal Disease Laboratory (Ames, Iowa 50010) for serotyping (Heddleston et al. 1972*a,b,* Brogden and Rhoades 1983). The culture was streaked on Bacto dextrose starch agar (DSA) fortified with 1% Bacto-PPLO serum fraction (SF) (Difco Laboratories Inc., Detroit, Mich. 48232). After 24-hours incubation at 37 C, the colonies were observed with a stereomicroscope (Olympus Corp. of America, New Hyde Park, N.Y. 11040) with obliquely transmitted light. All colonies were fluorescent, circular, smooth, convex, and glistening (Heddleston et al. 1964). A single colony was inoculated into 100 ml Bacto brain heart infusion broth (BHI) containing 1% SF. After 24-hours incubation, the growth was checked for purity and 1.0-ml aliquots were prepared for storage in liquid nitrogen. The *P. multocida* isolate was designated as the B-2 culture and was used throughout the study.

Challenge Culture

A 1:10 dilution of a 24-hour BHI broth culture of the B-2 isolate was used for challenging. Each bird was injected subcutaneously (SQ) in the dorsal anterior neck region with 0.2 ml of the culture (approximately $1.6–8.0 \times 10^7$ organisms by direct plate count).

Bacterin

One of the B-2 aliquots was inoculated into 1 liter BHI broth and incubated for 24 hours. At that time, the concentration of viable cells was estimated by direct plate count. The culture was checked for purity and inoculated into each of 3 laboratory mice (Hsd: [CF-1] BR white mice, Harlan Sprague-Dawley Farms, 3134 Seminole Highway, Madison, Wis. 53711) (0.2 ml intraperitoneally, IP) to test for virulence. Then 0.5% beta-propiolactone (O'neal, Jones, and Feldman, St. Louis, Mo. 63631) was added to the flask to inactivate the culture. The flask was reincubated at 37 C for 4 hours to hasten the death of *P. multocida* and disintegration of the beta-propiolactone. The killed broth culture (B-2 BB) was inoculated into BHI and thioglycollate broths, streaked onto DSA and MacConkey plates, and injected into each of 3 laboratory mice to verify complete inactivation.

An emulsified bacterin (B-2 OE) was prepared by mixing 500 ml of the inactivated B-2 BB with 482 ml Marcol 52 (Exxon Company, U.S.A., P.O. Box 66, Linden, N.J. 07036) and 25 ml Arlacel 80 (ICI United States, Inc., Wilmington, Del. 19801). The mixture was homogenized for 2 min in a Waring blender (Dynamics Corp. of America, New Hartford, Conn. 06057) at high speed. The preparation was stored at 4 C. The formulation of the incomplete adjuvant and use of beta-propiolactone were modifications of procedures for preparing poultry vaccines reported by other researchers (Heddleston and Hall 1958, Chute et al. 1962, Matsumoto and Helfer 1977, Stone et al. 1978).

The U.S. Department of Agriculture Veterinary Services Biologics Division granted National Wildlife Health Laboratory (NWHL) a permit to ship the experimental B-2 bacterin to Nebraska for a field trial.

Experimental Birds

Captive or wild-trapped giant Canada geese (*Branta canadensis maxima*) were used as surrogates for

Table 2. Challenge results for Experiment 2, geese vaccinated with broth bacterin without adjuvant (B-2 BB). Second dose with adjuvant (B-2 OE) given 7 or 11 weeks after first dose.

Treatment	Booster dose (weeks after first dose)	Challenge (weeks after first dose)	No. survivors / No. challenged
Vac (B-2 BB)	—	7	2/2
NT[a]			0/2
Vac (B-2 BB)	B-2 OE (7)	11	5/5
NT			0/5
Vac (B-2 BB)	B-2 OE (11)[b]	18	4/4
NT			0/5
Totals			
Vac			11/11
NT			0/12

[a] No treatment—control geese.
[b] One goose died from *Cloacotaenia megalops* parasitism 1 week after the B-2 OE vaccination.

Aleutian Canada geese. Captive birds were provided by the Nebraska Game and Parks Commission from their Sacramento-Wilcox Management Area flock. The adult breeders ranged from 6 to 16 years of age. The entire flock of 219 birds was test bled, and tracheal and cloacal swabs were collected 1 year before this work was started. Two groups of 24 adult captive geese (12 males each) were brought to Madison. One group (Table 1, Experiment 1) was used for testing the potency of the B-2 culture and the second group (Tables 1 and 2, Experiment 2) was used for testing bacterin efficacy. Eighteen domestic mallards (*Anas platyrhynchos*) and 4 American coots (*Fulica americana*) trapped on the Horicon National Wildlife Refuge were used for susceptibility and transmission studies of the B-2 isolate in Experiment 2 (Table 1). A group of 25 wild giant Canada geese were trapped at the Bay Beach Wildlife Sanctuary at Green Bay, Wisconsin, and placed in a semi-isolation unit. The geese were test bled, and tracheal and cloacal swabs were collected on the same day. The geese were observed for 1 month before vaccination to test long-term bacterin protection (Tables 1 and 3, Experiment 3). The remaining 171 geese in the Nebraska captive flock were used for a field trial to test the bacterin efficacy under natural conditions (Table 1, Experiment 4).

Serology

The agar-gel precipitation test and National Animal Disease Laboratory reference antigens (Types 1, 3, 4, and 5) were used for evaluating the pre- and post-challenge sera for antibodies (Heddleston et al. 1972*b*).

Table 3. Challenge results for Experiment 3, geese vaccinated with simultaneous doses of broth bacterin with (B-2 OE) and without adjuvant (B-2 BB).

Challenge (months after vaccination)	No. survivors/No. challenged	
	Vaccinates	Controls
4	2/2	0/2
6	2/2	0/2
8	2/2	0/2
10	2/2	0/2
12[a]	3/4	0/4
Total	11/12	0/12

[a] One vaccinated goose died 11 months after vaccination, with no significant lesions at necropsy (R. K. Stroud, pers. commun., 1983).

Experimental Design

Experiment 1.—A 24-hour BHI broth culture was serially diluted through 10^{-8}. The 10^{-4} through 10^{-8} dilutions were injected either intramuscularly (IM) into the breast muscle of the geese or SQ in the dorsal anterior neck region with 2 birds/dilution and 0.2 ml/dose.

Experiment 2.—Twelve geese were vaccinated with the B-2 BB in Nebraska. These birds, together with an equal number of untreated controls, were brought to Madison 7 weeks later (Table 2). Two vaccinates and 2 controls were then challenged with the homologous culture. Five of the 10 remaining vaccinates were given a booster dose of the B-2 OE (7 weeks post-vaccination, PV). Four weeks later, the vaccinates and 5 controls were challenged. The last 5 vaccinates were injected with the B-2 OE (11 weeks PV) and were challenged 7 weeks later along with 5 control geese. Surviving vaccinates were rechallenged with each successive group.

Four control American coots and 3 mallards were intermingled with the first group of challenged geese and shared the same feed and water. The remaining 15 mallards were placed in cages in the same isolation room. The 3 mallards from the floor and 3 from the cages were challenged along with the second group of vaccinated and control geese. Two additional unchallenged caged mallards were placed on the floor. These birds were then challenged along with the third group of geese 7 weeks later. All of the surviving geese, mallards, and coots were observed for 5 months after the last group was challenged before terminating the experiment.

Experiment 3.—Thirteen of the 25 geese from Green Bay were vaccinated. Each bird was inoculated with 0.5 ml each of the B-2 BB and B-2 OE SQ in the dorsal anterior neck region at 2 separate sites. Twelve geese were left as untreated contact controls. Two vaccinates and 2 controls were challenged every 2 months beginning 4 months PV (Table 3). Four birds remained to be challenged in both groups for the last challenge 12 months PV. Surviving vaccinates were rechallenged

with each successive group. Survivors were killed 2½ months after the 12-month challenge.

Experiment 4.—The 171 geese remaining in the captive flock were used for a field trial of the experimental vaccine. All except 14 of these birds were injected with 0.5 ml of B-2 BB in February. Eight weeks later, these birds were injected with 0.5 ml of B-2 OE approximately 6 weeks before the spring avian cholera die-off was expected to begin in the Nebraska Rainwater Basin, an annual occurrence since 1975 (Zinkl et al. 1977*b*). The captive flock, maintained in open pens frequented by free-flying waterfowl (Zinkl et al. 1977*a*), is naturally exposed to avian cholera.

RESULTS

Experiment 1.—The LD_{50} for the SQ inoculation route was $5 \times 10^{-7.0}$ and for the IM route, $5 \times 10^{-7.5}$ (Reed and Muench 1938).

Experiment 2.—All vaccinated geese survived challenge (Table 2). The controls died ≤24 hours post-challenge (PC) with 1 exception that died 68 hours PC. Surviving vaccinates rechallenged with each successive group of geese lived.

The 4 coots and 10 mallards held in contact with the challenged geese survived, whereas the mallards challenged with the second and third groups of geese died. *P. multocida* were not recovered from any tissues collected from the surviving sentinel mallards and coots, nor from any surviving vaccinated geese. Drinking water, feed, and fecal droppings were cultured and were also negative for *P. multocida.*

Experiment 3.—One of the 13 vaccinated geese died 11 months PV but before challenging (Table 3). Tissues were cultured and no bacterial pathogens were isolated. The 8 vaccinates survived challenge at 4, 6, 8, and 10 months PV. The 4 remaining vaccinates were challenged 12 months PV. Unfortunately, 1 goose was injured at the time of challenge and died 51 hours PC. Avian cholera organisms were injected through the neck muscle into the spinal column when the bird accidentally moved. The other 3 vaccinates survived the challenge. All controls died after challenge.

Surviving vaccinates were rechallenged at each successive exposure; the 4-month survivors were challenged a total of 5 times with the B-2 culture.

Inappetence and droopiness for 2–3 days following challenge were the only clinical signs observed in the vaccinates challenged in Experiments 2 and 3.

Attempts to isolate *P. multocida* from tracheal and cloacal swabs collected from challenged surviving vaccinated geese failed. Isolations were not made from any of the tissues when the birds were necropsied at the end of the study.

Serology.—Serum of 1 bird reacted positively with Types 3 and 4 antigens when the Nebraska captive flock was tested the year prior to this study. That bird was not used for this study. When the geese were brought to Madison for use in Experiment 2, only 1 vaccinate tested positively with Type 5 precipitins and remained positive throughout the study. Type 1 precipitating antibodies appeared in that bird's serum after the first challenge (7 weeks PV) and were present 1 day after the second challenge (11 weeks PV) but had disappeared by the third day and were not present 7 weeks later. Antibodies were again present 9 weeks after the third challenge (27 weeks PV). Five other vaccinates had either Type 1, Type 5, or Types 1 and 5 precipitins after either the booster vaccination or challenge. The other 6 vaccinates remained serologically negative throughout the study as did control birds.

Three of 13 vaccinated geese in Experiment 3 were serologically positive when tested before being challenged. One with Types 1 and 4 precipitating antibodies survived the challenge, but developed a necrotic enteritis and died 6 weeks PC. The second bird positive for Types 1, 3, 4, and 5 precipitins prior to the 10-month challenge survived and was rechallenged 2 months later. The serum was negative when the experiment was terminated. The third bird had Type 1 precipitins prior to

challenge. That was the bird accidentally injured at challenging. We failed to demonstrate precipitins in the remaining vaccinates even though they all survived challenging.

Experiment 4.—Ten of the 157 geese in the Nebraska captive flock died during the 2 months following their February vaccination. Two died from avian cholera and 8 from other causes (hepatitis, myositis, necrotic enteritis, parasitism, and trauma). One of the 2 vaccinates that died was a male >12 years old and the other was a male >16 years old. A single dead wild Canada goose was found in the captive pen. The carcass was sent to NWHL and avian cholera was diagnosed as the cause of death. None of the contact controls died during that spring.

DISCUSSION

The B-2 *P. multocida* antigen in the broth bacterin stimulated early protection in the vaccinates. The slowly released antigen from the OE bacterin sustained this protection up to 12 months PV against challenge with overwhelming numbers of homologous organisms. The simultaneous use of bacterin, with and without adjuvant, eliminated the necessity of rehandling geese—an important consideration in the development of bacterin for Aleutian Canada geese, as there would be only 1 opportunity to vaccinate.

The 12-month protection of geese in this study is comparable to that demonstrated by Heddleston and Reisinger (1959, 1960). They reported 12 of 14 (92%) chickens were protected 54-weeks PV with 1 OE bacterin injection and 78 of 90 (86.7%) chickens were protected 52-weeks PV with 1 dose of aluminum hydroxide aqueous absorbed (AAA) vaccine. A shorter protection period was reported by Singer et al. (1977). In that study, 11 of 15 (73.4%) domestic geese vaccinated with 2 doses of autogenous AAA bacterin survived challenge 3 weeks PV. Queen and Quortrup (1946) reported 4 of 5 wild mallards vaccinated with 2 doses of a heated (50 C) plate culture suspension of a field avian cholera isolate survived challenge 1-week PV, the first reported use of an avian cholera bacterin in wild mallards.

The composition of the adjuvant for the B-2 OE resulted in the formation of a stable product. The oil and aqueous phases remained homogeneous after storage for 20 weeks at 4 C. We detected no tissue reaction to the adjuvant at the vaccination site in the geese.

We found no evidence that *P. multocida* spread from challenged geese to susceptible coots and mallards placed in contact with them, or any indication that challenged survivors were carriers. Quortrup et al. (1946) and Simensen and Olson (1980) both reported that avian cholera was transmitted from infected birds to penmates by aerosol.

The B-2 culture was highly virulent for the unprotected challenged controls used in this study as was the snow goose isolate used by Wobeser et al. (1979).

The presence of Types 1, 3, 4, and 5 precipitins in the sera of some vaccinates before challenging indicated that capsular antigens were in the bacterin in addition to the somatic Type 1 antigen. Appearance of precipitating antibodies and the immune status of individual birds did not appear related. Both seropositive and negative vaccinates resisted challenge as reported in other studies (Singer et al. 1977).

The death of the single vaccinate from the accidental injection of *P. multocida* into the nervous system shows the immune system of a protected bird can be overcome if the organisms are injected into a susceptible site. Ideally, experimental challenge should simulate a natural exposure to avian cholera without overwhelming the bacterin efficacy in the test birds, a constant problem through the years in the development of avian cholera bacterins.

MANAGEMENT IMPLICATIONS

This vaccine was shown to be efficacious and safe to use in both captive and wild-trapped giant Canada geese with unknown history of previous exposure to naturally occurring pathogens. The results suggested the usefulness of the vaccine for immunizing Aleutian Canada geese and other endangered species. Administration of a single dose of vaccine could easily be coordinated with banding programs for free-flying species. The use of this bacterin should be considered for birds being raised for propagation on refuges, game farms, and zoos frequently experiencing exposure to avian cholera in wild birds attracted to these sites.

SUMMARY

A broth bacterin was prepared from a Type 1 *Pasteurella multocida* culture isolated from a lesser snow goose that died of avian cholera in the Central Valley of California. Captive giant Canada geese vaccinated with either the broth bacterin alone or together with a booster dose of oil emulsion (OE) vaccine survived a challenge exposure to the homologous culture 4–7 weeks after vaccination. Equal numbers of untreated control geese died within 24 hours after exposure to the challenge culture.

Wild-trapped giant Canada geese were injected simultaneously with a single dose each of broth and emulsified bacterins. Two vaccinates and 2 untreated contact controls were challenged every 2 months, 4–12 months post vaccination. All except 1 vaccinate survived challenge with live homologous cultures whereas all untreated control geese died. *P. multocida* were not recovered from any of the surviving vaccinates; moreover no apparent transmission from challenged geese to closely associated coots or mallards sharing the same water and feed was observed.

A small flock of captive giant Canada geese were vaccinated with the experimental bacterin in Nebraska to test its efficacy under field conditions. Only 2 of 157 vaccinates died from avian cholera during an annual spring die-off.

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HUNTING PRESSURE ON BLACK-BELLIED WHISTLING-DUCKS AS INDICATED BY EMBEDDED SHOT

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Hunting pressure on waterfowl normally is estimated by analyses of band recoveries. However, those methods are precluded for such species as the black-bellied whistling-duck (*Dendrocygna autumnalis*), in which few birds are banded. Alternately, Elder (1950, 1955) demonstrated that a close relationship between the band-recovery analyses then in use and the incidence of embedded shot in live birds offered another means of estimating

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