The Duration of Immunity to an Inactivated Adjuvanted Canine Parvovirus Vaccine. A 52 and 64 Week Postvaccination Challenge Study

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SUMMARY

Dogs were successfully isolated for a period of either 52 or 64 weeks following vaccination with an inactivated, adjuvanted canine parvovirus-2 vaccine. Antibody persisted in all ten vaccinated dogs, although in one case by 52 weeks postvaccination only virus neutralizing antibody, and not hemagglutination-inhibiting antibody, could be detected. Sentinel unvaccinated dogs housed alongside the vaccinated dogs throughout the study remained free of canine parvovirus-2 antibody until challenged. Upon oral challenge with canine parvovirus-2 infected material all unvaccinated dogs developed one or more signs of canine parvovirus-2 disease, shed virus and developed antibody. None of the vaccinated dogs became overtly sick. Of the five vaccinated dogs challenged 52 weeks after vaccination, three shed virus and one showed a significant rise in antibody. At 64 weeks after vaccination only one of the five challenged dogs shed virus and showed a boost in antibody titer.

RÉSUMÉ

Recherche de la durée de l'immunité contre la parvovirose canine, au moyen d'une infection de défi réalisée au bout de 52 et 64 semaines après l'administration d'un vaccin inactivé et doté d'un adjuvant

Les auteurs réussirent à isoler avec succès dix chiens, pour une période de 52 à 64 semaines, après leur avoir administré un vaccin inactivé contre le parvovirus-2 canin, auquel ils avaient incorporé un adjuvant. Des anticorps persistèrent chez tous ces chiens mais, dans un cas, au bout de 52 semaines, on pouvait encore détecter des anticorps neutralisants, mais pas d'anticorps inhibiteurs de l'hémagglutination. Les chiens témoins occupaient les mêmes locaux que les vaccinés et ils ne développèrent d'anticorps contre le parvovirus qu'après l'infection de défi, par la voie buccale; ils manifestèrent aussi un ou plusieurs des signes de la maladie due au parvovirus-2 canin et ils éliminèrent de ce virus. Aucun des chiens vaccinés ne développa de parvovirose clinique. Trois des cinq chiens soumis à l'infection de défi, 52 semaines après leur vaccination, éliminèrent du virus; l'un d'entre eux afficha aussi une élévation significative de son titre d'anticorps. Par ailleurs, un seul des cinq qui subirent l'infection de défi, 64 semaines après leur vaccination, élimina du virus et afficha une élévation de son titre d'anticorps.

INTRODUCTION

In the prophylaxis of canine parvovirus-2 (CPV-2), inactivated feline panleukopenia virus (FPV) vaccines were the first to be widely used. They conferred relatively short lived immunity. Detectable hemagglutination-inhibition (HI) antibody was not present three months after vaccination (1). Live modified vaccines containing FPV have been said to be capable of producing an antibody response persistent up to 12 months (2). They have the disadvantages of being live and containing heterologous virus, which may fail to produce satisfactory protection in some vaccinated dogs (3, 4, 5, 6).

More recently homologous CPV-2 vaccines, including live modified and killed, have become available. However simple, nonadjuvanted killed CPV-2 vaccines have also been found to have short lived immunity. Vaccinated dogs could be infected when challenged as early as 12 weeks after vaccination (1).

This report describes the duration of immunity study on an inactivated, adjuvanted CPV-2 vaccine, the development of which has been reported (5).

MATERIALS AND METHODS

Dogs

A total of 14 cross-bred dogs, born to antibody free bitches and themselves free of antibody to CPV-2 (reciprocal HI titer <2), were obtained. The animals, between eight and 12 weeks of age, were housed in individual cages. When the dogs grew larger, they were moved to small pens. Two rooms were used for the cages and the pens, and the dogs were randomly assigned to them. The wearing of protective clothing and disinfection of personnel were strictly adhered to throughout to prevent accidental introduction of parvovirus. Guidelines of the Canadian Council on Animal Care were followed throughout the study.

Vaccines

An inactivated, adjuvanted CPV-2 vaccine, the development of which has been described previously (5), was obtained (serial 12A).[1]

Virology and Serology

Virological and serological proce-

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[1]Pavlan-C, Langford Laboratories, Guelph, Ontario.

dures have been described previously (5, 7). Sera were assayed for CPV-2 HI antibody using four hemagglutinating units of CPV-2 (strain Rae) and African green monkey red cells at 4°C. Some sera were also assayed for virus neutralizing (VN) antibody using 100 $CCID_{50}$ of CPV-2 and a dog tumor cell line designated A-72(8).

Experimental Design

Ten dogs received the vaccine. All vaccinations were a 1 mL dose given either subcutaneously or intramuscularly (Table I) and repeated after two weeks. Four dogs remained unvaccinated as controls and were housed in the same two rooms as the vaccinated dogs. Blood samples were collected at least monthly for serology.

Fifty-two weeks after initial vaccination, five vaccinated dogs and three nonvaccinated dogs were moved to a separate isolation room. These dogs were inoculated orally with 5 mL of 10% w/v gut mucosal homogenate in phosphate buffered saline. The gut mucosa had been collected from a dog five days after experimental infection with CPV-2. The challenge dose contained $10^{5.1}$ $CCID_{50}$ of CPV-2. The dogs were denied food for 24 hours before and 24 hours after challenge. They were monitored daily over a 12 day postchallenge period for signs of alimentary tract disturbance, fever, reduction in appetite, or other clinical signs of disease. Fecal samples were collected for virus recovery.

The remaining five vaccinated and one nonvaccinated dog continued to be housed together in isolation until 64 weeks after vaccination when they too were challenged and monitored as above.

RESULTS

The serological results as determined by hemagglutination-inhibition tests are shown in Table I. The integrity of the isolation housing is demonstrated by the continual freedom from CPV-2 antibody of the unvaccinated dogs up until the time they were challenged. The antibody response in vaccinated dogs was at a peak three weeks after initial vaccination (one week after the second vaccination). Titers then decreased, often sharply, by eight or 16 weeks. Thereafter decline was either very slow or inapparent. After an initial good response (reciprocal HI titer = 192 at three weeks), dog W6 had an HI titer of 32 which was the lowest of the group at eight weeks and at 52 weeks it had a titer of < 8. When this dog was tested at 64 weeks the reciprocal VN titer was 16 and the HI titer was still < 8.

The clinical response to the challenge 52 weeks after initial vaccination was as follows: four of the five vaccinated dogs remained free of any fever, alimentary disturbances or other clinical signs. One of the vaccinated dogs (W1) had a mild diarrhea on day 6 postchallenge. All three unvaccinated dogs lost appetite, and two of them developed profuse diarrhea. One of these dogs with diarrhea had fever ($> 39.5°C$) for two days and vomited during one day.

Four of the five vaccinated animals challenged at 64 weeks remained free of any clinical signs. The fifth dog (W6) in this group and the unvaccinated dog (SM7) remained clinically normal except that each developed a transient fever ($> 39.5°C$) on days 6 and 4 postchallenge respectively.

The recovery of CPV-2 from feces of challenged dogs is shown in Table II. Three of the five vaccinated dogs challenged at 52 weeks and one of the five vaccinates challenged at 64 weeks shed virus after challenge, but in two of these animals, levels of virus were low and were only recovered after blind passage of inoculated cell cultures. All four nonvaccinated dogs shed virus of which three had virus detected on initial culture of inoculated cells.

The serological response to challenge (Table I) indicates absence of antibody rise in all but two of the vaccinated dogs. These two showed marked increases over the ten day postchallenge period. Dog W6 reached a reciprocal titer of 8 192 by 15 days postchallenge. All nonvaccinated dogs had postchallenge titers of 192 to > 512.

TABLE I
SEROLOGICAL RESPONSE (RECIPROCAL HI TITER) TO VACCINATION WITH AN INACTIVATED, ADJUVANTED CPV-2 VACCINE AND CHALLENGE

			Weeks after initial vaccination									
Group[a]	Route[b]	Dog	0	2	3	8	16	34	52	(10 dpc)[c]	64	(10 dpc)[d]
Vaccinated	SC	R1	<2	48	128	64	48	24	24	16		
	IM	W1	<2	128	96	64	64	96	64	96		
	SC	R5	<2	32	256	192	48	24	48	>512		
	IM	W5	<2	96	256	96	48	24	24	16		
	SC	SM2	<2	256	1024	256	128	192	192	192		
	SC	R2	<2	96	192	64	48	24	48		48	32
	IM	W2	<2	96	64	64	64	48	64		64	48
	SC	R6	<2	96	768	192	96	64	96		64	64
	IM	W6	<2	192	192	32	12	16	<8		<8	256
	SC	OH2	<2	256	768	96	48	24	24		24	16
Nonvaccinated		R7	<2	<8	<8	<8	<8	<8	<8	>512		
		R8	<2	<8	<8	<8	<8	<8	<8	512		
		W7	<2	<8	<8	<8	<8	<8	<8	>512		
		SM7	<2	<8	<8	<8	<8	<8	<8		<8	192

[a]Vaccinated dogs received two doses of 1 mL of inactivated, adjuvanted CPV-2 vaccine two weeks apart.
[b]IM = intramuscular, SC = subcutaneous vaccination.
[c]Ten days postchallenge at one year after vaccination.
[d]Ten days postchallenge at 15 months after vaccination.

DISCUSSION

Canine parvovirus-2 is very infectious and like other parvoviruses, is able to survive for at least six months in the environment (9). These facts have made it difficult for satisfactory and controlled duration of immunity studies. A previous duration of immunity study with this vaccine was invalidated by contamination after nine months (5). The success of isolation procedures in the present study were demonstrated by the absence of specific antibody in the sentinel dogs at 52 and 64 weeks.

Antibody to CPV-2 persisted in all the vaccinated dogs until they were challenged at 52 or 64 weeks. The vaccinated dog which did not have detectable HI antibody at 52 weeks still had detectable VN antibody at 64 weeks. This persistence of antibody is in marked contrast to previously reported duration of immunity following inactivated CPV-2 vaccination when studies have been strictly controlled (1). With inactivated FPV and CPV-2 vaccines the HI titer in most vaccinated dogs was equal to or less than the minimum limit of detection (≤ 10) by 12 weeks after vaccination (1).

The persistence of immunity conferred by this inactivated, adjuvanted CPV-2 vaccine as demonstrated by the continued detection of antibody was confirmed by the challenge experiments. At 52 weeks after vaccination only one of the five challenged dogs showed any clinical sign. This was a transient mild diarrhea and the lack of CPV-2 in stool samples and the absence of any antibody boosting in the postchallenge period in that dog, suggest the diarrhea was unrelated to CPV. However three of the five vaccinates in this group did shed virus following challenge. In only one dog was virus recovered at the initial isolation attempt and this dog did have a significant increase in antibody titer from 48 to > 512 following challenge. The other four dogs showed no such boosting which indicates that virus invasion did not occur. Four of the five dogs challenged 64 weeks after vaccination completely resisted the challenge. They did not shed virus in the feces and their antibody titers were not boosted. The fifth dog in the group developed a transient fever, shed virus and did show an antibody rise but no overt sign of illness was detected.

These results are much more satisfactory than those obtained with the various inactivated vaccines tested previously (1). Six of six dogs that were challenged five to ten weeks after inactivated FPV vaccine inoculation, and two of two dogs challenged 12 weeks after vaccination with an inactivated CPV-2, shed virus and showed a 30-500 fold increase in antibody titer after challenge.

The explanation for the duration of immunity with the presently tested vaccine is unlikely to be due to the antigen content. The vaccine serial used had an antigen content prior to inactivation equivalent to 2 304 HA units per 1 mL dose. This is only approximately 11% of the antigen content of an inactivated CPV-2 vaccine previously investigated (1). The adjuvants in the vaccine used in our study probably were essential in stimulating the prolonged immunity. The specific role of these adjuvants such as enhanced stimulation of memory lymphocytes or sustained slow release of antigen from depots is to be investigated.

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TABLE II
RECOVERY OF CPV-2 FROM FECES OF CHALLENGED DOGS

Group	Weeks Postvaccination	Dog	Days Postchallenge 0	4	5	6	7	8	9
Vaccinated	52	R1	–	+	+	+	+	+	NS
	52	W1	NS	–	–	–	NS	NS	NS
	52	R5	NS	–	–	++	++	++	NS
	52	W5	NS	–	+	+	–	–	NS
	52	SM2	–	–	–	–	–	–	NS
Vaccinated	64	R2	–	–	–	–	–	–	–
	64	W2	–	–	–	–	–	–	–
	64	R6	–	–	–	–	–	–	–
	64	W6	–	–	+	+	+	++	++
	64	OH2	–	–	–	–	–	–	–
Nonvaccinated	52	R7	–	+	+	+	NS	NS	–
	52	R8	NS	NS	++	++	++	++	NS
	52	W7	–	+	+	++	++	NS	–
	64	SM7	–	–	+	+	++	+	++

+ = Virus only detected after passage of inoculated cell cultures.
++ = Virus detected on initial culture of inoculated cells.
NS = No sample available.

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LETTERS TO THE EDITOR

Observation on Shipping Fever

DEAR SIR:

A recently published bulletin, Control of "Shipping Fever" in Cattle (Can Vet J 24(4): xiii, 1983), carries an implication of impartiality as a result of its source, Agriculture Canada. In our view several of the points made therein stem more from the particular objectives of researchers at Animal Diseases Research Institute, Lethbridge than from an overview of currently available information. The following are our observations regarding the above mentioned bulletin and "shipping fever" in general.

1) While preinfection of cattle with infectious bovine rhinotracheitis (IBR) virus clearly enhances susceptibility to pasteurella related pneumonia in laboratory trials, the frequency of IBR infection as a predisposing factor in field cases of "shipping fever" pneumonia is unknown. It seems safe to assume that it is not a universal predisposing event and it may be infrequent.
2) *Pasteurella multocida* is isolated much less often from bovine pneumonic lungs than is *P. haemolytica* serotype 1 which occurs in up to 90% of cases of "shipping fever" pneumonia (4,5).
3) Although the bulletin indicates that *P. multocida* is the only agent which has by itself induced pneumonia it should be recognized that *P. haemolytica* has reliably induced pneumonia in calves when infused into the lower airways or injected directly into the lung. At the present time this is widely accepted and used in North America and Europe as a means of inducing pneumonic pasteurellosis for investigation of pathogenesis and host resistance (1, 2,3,6).
4) The bulletin indicates that aerosol vaccination with *P. haemolytica* fails to induce protection. Published reports of research suggest the opposite and serological evidence indicates that immune response induced by field exposure to *P. haemolytica* correlates positively with resistance to "shipping fever" pneumonia (1,3).
5) Although it is indicated that the findings described in the bulletin "complement research at the Ontario Veterinary College" (OVC) it may not be clear to readers that faculty, staff and graduate students at OVC have been, and will continue to be, actively in pursuit of advanced efficacious immunizing agents for prevention of pneumonic pasteurellosis due to *P. haemolytica* in cattle.

It is important that investigation of animal disease continue to involve several groups of researchers with resultant "insurance" derived from the diversity of hypotheses generated in relation to specific problems. Utility of a given model may advance understanding but with all the best intentions the laboratory scientist cannot precisely duplicate field conditions. For this reason, the ultimate proof of pathogenesis or of vaccine efficacy will come from studies of spontaneous disease. These studies will be based upon, and follow from, laboratory trials of the kind conducted in several Canadian laboratories.

Sincerely yours,

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Mesangiocapillary Glomerulonephritis in Finn Cross-bred Lambs

DEAR SIR:

Recently, lambs with mesangiocapillary glomerulonephritis (membranoproliferative glomerulonephritis) have been found in three Finn-cross flocks in northern Alberta. These cases are the first to be recognized in Canada. Previously, the disease was confined to a flock of Finn breeding at the Moredun Research Institute in Edinburgh, Scotland. The disease is thought to be inherited in a recessive mode. Affected lambs die before four months of age, and may not have clinical signs.

Histological and electron microscopic examination of kidneys are required for a definitive diagnosis. It is important to recognize affected flocks in order to determine the prevalence of the disease in Canada.

Yours truly,

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