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Influence of maternally-derived antibodies in 6-week old dogs for the efficacy of a new vaccine to protect dogs against virulent challenge with canine distemper virus, adenovirus or parvovirus

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ABSTRACT

The results from three studies determining the efficacy of a canine multivalent vaccine in the presence of maternal antibodies are reported. Each study used 15 six week old dogs; five dogs were sero-negative; the remaining 10 had maternally derived antibodies to CDV, CAV and CPV. The five MDA-negative dogs and five of the MDA-positive dogs were vaccinated twice with the vaccine while the remaining 5 MDA-positive dogs were administered sterile water. According to EU guidelines for MDA studies dogs were challenged when maternally-derived antibodies in non-vaccinated dogs had greatly diminished or disappeared (3–5 weeks after second vaccination); clinical observations and rectal temperatures were recorded, and sera and faecal samples (CPV study only) were collected throughout the study.

After challenge, non-vaccinated dogs showed clinical signs of infection while none of the vaccinated dogs did. MDA-negative vaccinated dogs sero-converted with increases in titre observed after each vaccination, and further increases observed after challenge. MDA-positive vaccinated dogs showed declining antibody titres following the first vaccination, but increases after the second vaccination and further increases after challenge. In all vaccinated dogs the immune responses generated were protective, irrespective of the presence of maternal antibodies, as demonstrated by heterologous viral challenge.

In conclusion, two doses of the DHPPi/L4R vaccine administered to dogs from six weeks of age in the presence of maternal antibodies aided in the protection against virulent challenge with CDV, CAV-1 or CPV.

Introduction

For over a decade canine vaccines have been categorised into core, non-core and non-recommended groups [7], with canine distemper, parvovirus and adenovirus considered core vaccine components. These categories have been further developed and currently form the basis of the World Small Animal Veterinary Association (WSAVA) Guidelines for the Vaccination of Dogs and Cats [16].

Active immunisation of dogs is essential at reducing the risk of contracting infectious diseases and in particular viral infections [3]. As vaccine administration has become more routine and widespread, the incidence of most commonly observed diseases has been reduced, although there are occasional outbreaks in vaccinated animals [10]. Vaccine technology has also evolved in line with new disease agents to provide improved performance or broader efficacy [1].

Maternally derived immunity is considered the primary cause of vaccine failure in young dogs [6,8,12]. To overcome interference by maternally derived antibodies (MDA), and ensure protection when maternal antibody levels wane, it is recommended to vaccinate puppies repeatedly between 6 and 16 weeks of age. However, this is logistically demanding and most products now have a single or double vaccination regimen depending on the age of dog and whether MDA are expected to be present. Although MDA was thought to have more of an impact on live vaccine components, recent studies have demonstrated that maternal antibodies can

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have a negative influence on the generation of immune responses following administration of vectored [11] or adjuvanted killed antigen vaccines [14,15]. What these studies, and others in dogs [2] have shown is that the level of maternal antibodies has a significant influence on the ability of a vaccine to generate a passive immune response, with decreasing maternal antibody levels permitting more response from the vaccine.

In this paper we describe the efficacy of a new multivalent vaccine (DHPPi/L4R), containing the core viral components, when administered to dogs from six weeks of age in the presence of maternally derived antibodies. Dogs were challenged following vaccination and the impact of vaccination on clinical variables and serology was examined by comparing MDA-positive and MDA-negative vaccinated dogs to MDA-positive non-vaccinated dogs in each study.

Materials and methods

This study reports the results of three European Pharmacopeia monograph compliant trials investigating the efficacy of canine distemper virus (CDV; monograph 01/2008:0448), canine adenovirus type 2 (CAV-2; monograph 01/2008:1951) and canine parvovirus (CPV; monograph 01/2008:0964) in a new multivalent vaccine for dogs. The studies were conducted in accordance with the Act on Animal Health and Animal Welfare of The Czech Republic, and had been approved by Bioveta a.s. and Zoetis ethical review committees. Day 0 was when dogs received the first administration of vaccine or sterile water.

Animals

In each study fifteen 6-week old Beagle dogs were enrolled. Five dogs were sero-negative (MDA−) with the remaining ten dogs, randomly allocated to two groups, being sero-positive to CDV, CPV, CAV-1 and CAV-2 (MDA+), at titres comparable to those observed in the field; MDA titres prior to first vaccination are shown in Table 1. The MDA-negative pups were derived from SPF dams, while the MDA-positive pups were derived from conventional health status dams which had been vaccinated with the test vaccine described below. All dogs were of good general health and free from infection with the viral agents contained within the vaccine. Dogs from the MDA− group and one MDA+ group were vaccinated with DHPPi/L4R. Dogs from the second MDA+ group served as controls and were administered sterile water. After the second test material administration, all vaccinated dogs (MDA− and MDA+) and two non-vaccinated dogs (MDA+), with greatly diminished or no MDAs and selected at random by a company biometrician, were challenged with the relevant challenge virus.

Vaccine

An experimental vaccine batch was produced which contained modified live CDV, canine parainfluenza virus (CPiV), CAV-2, CPV-2b (DHPPi), inactivated *Leptospira interrogans* serovars Canicola, Icterohaemorrhagiae and Bratislava, *Leptospira kirschneri* serovar Grippotyphosa, and rabies virus (L4R). The DHPPi component was freeze-dried while the L4R component was a liquid containing adjuvant (aluminium hydroxide). The control product was sterile water. Administration (1 ml) was by the subcutaneous route behind the left shoulder blade on day 0 and behind the right shoulder blade on day 21, using standard aseptic technique.

Challenge

CDV isolate Snyder Hill was obtained from the American Type Culture Collection (ATCC); the CAV-1 isolate Mirandola was obtained from the Animal and Plant Health Inspection Services, Centre for Veterinary Biologics (APHIS, CVB); and the CPV-2b isolate 212/98 was obtained from the University of Bari, Italy. For the CDV (10^{-1} dilution of an unknown titre virus stock) and CAV-1 ($10^{6.3}$ $TCID_{50}$/mL) studies 1 mL of challenge material was administered by the intravenous route on day 42; for CPV ($10^{6.6}$ $TCID_{50}$/mL) a 2 mL dose was administered with 1 mL orally and 1 mL intranasally (0.5 mL per nostril) on day 56. The day of CPV challenge was postponed by 2 weeks from day 42 to day 56, because the MDA+, non-vaccinated control dogs still had detectable maternal antibodies on day 28 and day 35.

Observations and samples

Rectal temperatures (°C) of all animals were recorded daily for a period of seven days after each vaccination, and from prior to challenge strain administration until the end of the study. Clinical observations were performed daily from day-2 until the end of the study. Additional general health observations were performed at least once daily at times distinct from clinical observations. Any dogs which exhibited signs of disease, such that in the opinion of the examining veterinarian their welfare was seriously affected, were euthanased as appropriate to avoid unnecessary suffering.

Table 1
Maternal derived antibody titres prior to first vaccination. All results shown are virus neutralisation titres where the end point was assessed as the serum dilution where more than 50% of the characteristic cytopathic effect was attenuated. Different groups of dogs were used in each of the three studies.

Animal	Treatment	Study one		Study two		Study three
		CAV-1	CAV-2	CPV-2	CPV-2b	CDV
1	MDA-negative; vaccinated	<2	<2	<5	<5	<2
2		<2	<2	<5	<5	<2
3		<2	<2	<5	<5	<2
4		<2	<2	<5	<5	<2
5		<2	<2	<5	<5	<2
1	MDA-positive; vaccinated	64	32	320	5120	4
2		64	64	80	640	8
3		64	128	320	640	4
4		⩾256	128	320	2560	2
5		64	64	640	2560	2
1	MDA-positive; controls	128	64	640	1280	8
2		128	128	160	1280	16
3		64	64	320	2560	8
4		128	128	160	5120	4
5		128	128	80	1280	8