Protection of puppies against canine herpesvirus by vaccination of the dams

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Six bitches free of canine herpesvirus 1 (CHV-1) were vaccinated against the virus; a first injection was given 10 days after the presumed date of mating and a second six weeks later. Six similar bitches were left unvaccinated as controls, and all the pups were challenged oronasally with a virulent strain of CHV-1 at three days of age. All the vaccinated bitches seroconverted and had high antibody titres when the puppies were challenged, but the control bitches remained seronegative. In the control group, 62 per cent (18 of 29) of the pups died of CHV-1-induced disease; most of them showed typical clinical signs and macroscopic lesions, and CHV-1 infection was confirmed by the isolation of the virus or by PCR. None of the puppies in the vaccinated group died of CHV-1 infection. The efficacy of the vaccine was confirmed in CHV-1-positive breeding units. The rate of pregnancy tended to be higher in vaccinated bitches and the mortality of pups before weaning was significantly reduced in the litters born to vaccinated bitches.

CANINE herpesvirus 1 (CHV-1) was first isolated in 1965 (Carmichael and others 1965), and it has since been shown to be enzootic in dogs all over the world (Reading and Field 1998, Rijsewijk and others 1999). The virus is a member of the Alphaherpesvirinae and is closely related to feline herpesvirus (FHV) and phocid herpesvirus (PHV) but the infection is restricted to the members of the Canidae family. It is associated with an acute and usually fatal infection of puppies during their first few weeks of life. The pups become infected oronasally during whelping or in their first days of life from the bitch or their littermates. In adult dogs the virus causes only a mild infection of the upper respiratory or genital tract (Hill and Mare 1974), but infection of the pregnant bitch may induce stillbirth, abortion and mortality among the pups (Hashimoto and others 1979, 1982, 1983). The virus is also strongly suspected to cause infertility. Like other Alphaherpesviruses, it may induce a latent infection in the trigeminal and lumbosacral ganglia and other tissues (Burr and others 1996, Miyoshi and others 1999). In the absence of specific treatment or preventive measures, a CHV-1 infection is usually fatal and may be responsible for heavy losses in some breeding units. This paper describes a study of the protection of puppies against CHV-1 by vaccinating their dams.

MATERIALS AND METHODS

Animals

Twelve litters born to 12 specific pathogen-free, CHV-1-free bitches were used. Six of the bitches were vaccinated and the other six were given a placebo consisting of the oily diluent of the vaccine (except for the first control bitch which did not receive a placebo). The bitches were given two subcutaneous injections six weeks apart, the first about 10 days after the presumed date of mating and the second about 10 days before the expected date of whelping. The bitches were allocated to the two groups at random.

Vaccine

The vaccine was a freeze-dried inactivated purified CHV-1 F205 strain fraction enriched with viral glycoproteins. The F205 strain was grown on Madin-Darby canine kidney (MDCK) cells, and the viral suspension was inactivated with ethylenimine, concentrated and purified. An antigen fraction enriched with CHV-1 glycoproteins was obtained by incubating the purified viral suspension with a non-ionic detergent and sedimenting the capsids by ultracentrifugation. The supernatant was collected and adjusted to an antigen concentration corresponding to 10^6 to 10^8 50 per cent cell culture-infective doses ($CCID_{50}$)/ml of CHV-1 before it was inactivated and freeze-dried. One dose of freeze-dried pellet was reconstituted with one dose of an emulsion for each injection.

Virulent challenge of puppies

The puppies were challenged three days after birth (day 3) with about 100 $CCID_{50}$ of a virulent CHV-1 strain (C4012SE French isolate) by inoculating 0·25 ml in each nostril and 0·5 ml orally. They were examined daily for three weeks, and then euthanased and examined postmortem.

Samples of kidney and lung were pooled for viral isolation on MDCK cells and by PCR. Samples of liver, spleen and brain were also included in the pool from litter 1, but the kidneys and lungs are the organs with the highest CHV-1 titres and are therefore the organs of choice. The samples were stored at –70°C until used. About 1 g of each organ was diluted 1/10 or 1/20 in culture medium enriched with antibiotics, homogenised and clarified by centrifugation. One aliquot of the supernatant was used to inoculate MDCK cells, and one aliquot was frozen at –70°C for PCR. The main organs were examined histologically when necessary.

CHV-1 neutralising antibodies

Blood samples were collected from the bitches at the first and second vaccine injection and on the day of challenge, and from the puppies on the day of challenge and at the end of the period of monitoring, and the sera were titrated for CHV-1-neutralising antibodies. Threefold serial dilutions of the sera were made in culture medium and transferred in quadruplicate to 96-well plates at 50 µl per well. Approximately 20 $CCID_{50}$ of CHV-1 in 50 µl were then added to each well and the plates were incubated at 37°C for one hour and overnight at 4°C. One hundred and fifty microlitres of a suspension of MDCK cells containing 100,000 cells/ml was then added to each well and the plates were incubated at 37°C for five days. The cell cultures were checked microscopically for the characteristic cytopathic effect of CHV-1. The serum neutralising titre was defined as the logarithm of the reciprocal of the highest serum dilution that inhibited the cytopathic effect in 50 per cent of the cell cultures.

ELISA anti-CHV-1 gB antibodies

ELISA microplates (Maxisorp; Nunc) were coated with a capture monoclonal antibody (mAb) specific to the gB glycoprotein of CHV-1 for 18 hours at 5°C and then rinsed. In dilution plates, 50 µl of each serum on test and controls were serially diluted in dilution buffer and a purified CHV-1 F205

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ıtigen was added to each serum dilution. The plates were ıcubated for 18 hours at 5°C. The serum/antigen mixture as transferred on to the ELISA plates coated with the capture ıtibody, incubated for three hours at 37°C and then rinsed. detection anti-CHV-1 gB mAb labelled with horseradish per-ıidase (HRP) was added to each well. After one hour at 37°C, ıe plates were rinsed and incubated with HRP substrate for) minutes at 20°C. The reaction was then stopped with 2M ılphuric acid and optical densities (OD) were read at 450 to 30 nm. The 50 per cent ELISA serum titre corresponded to the verse of the dilution of the serum on test which gave an OD 50 per cent (optical density equal to 50 per cent of the max-ıal optical density).

In vaccinated animals, the relationship between ELISA anti-HV-1 gB antibody titres (ELISA Ab) and CHV-1 neutralising ıtibody titres (VN Ab) has been shown to be:

$$\log_{10}[\text{VN Ab}] = 1{\cdot}046 \times \log_{10}[\text{ELISA Ab}] - 0{\cdot}42$$

olation of virus

ıe virus was isolated on a freshly established layer of MDCK lls in 25 cm² flasks. Cell monolayers were inoculated with nl of the supernatant of the ground organs. After one hour's ıtact at 37°C, the inoculum was discarded and replaced ch serum-free medium. The flasks were incubated at 37°C a damp atmosphere containing 5 per cent carbon dioxide r five days, and checked daily for any cytopathic effect spe-ic to CHV-1. If no effect was recorded at the first passage, a :ond passage on MDCK cells was carried out after freezing d thawing the cell monolayer of the first passage. The lysate ıs inoculated on to a monolayer of MDCK cells under the me conditions. The sensitivity of the viral isolation was tween 2 to 5 $CCID_{50}$.

:R

R was carried out on DNA extracted with the IsoQuick kit ficroprobe Corporation) from 50 µl of the supernatant ım the ground organs containing the cell fraction obtained centrifugation. The extracted DNA was reconstituted with µl of ultrapure water, and the PCR was applied to a 1 µl sam-, using 1·25 units of Taq DNA Polymerase (GibcoBRL) and imers allowing the amplification of a fragment of 548 cleotides of the gD gene. Forty cycles of 30 seconds of naturation at 95°C, 45 seconds of hybridisation at 55°C and seconds of elongation at 72°C were used in each PCR. The ɔduct obtained was identified by electrophoresis on 1 per ıt agarose gel; the presence of DNA of CHV-1 was detected by ·and corresponding to 548 nucleotides. It was shown that PCR could detect the equivalent of 0·01 $CCID_{50}$.

ıta analysis

e vaccinated and control groups were compared in terms mortality, the presence of specific lesions of CHV-1 infec-n postmortem, and evidence of CHV-1 either by viral iso-ion or by PCR by means of a chi-squared test and Fisher's ıct test (Statgraphics SGWIN3 software). All the puppies that d during the three weeks after they were challenged and l characteristic gross lesions were considered to have been ected by CHV-1. The isolation of herpesvirus and/or a pos-e PCR was considered as a confirmation.

ıld trial

hty-nine bitches of various breeds which had been bred in en breeding units infected with CHV-1 were used. The CHV-atus of the breeding units was determined on the basis of ological results (high seroprevalence and/or presence of ches with ELISA antibody titres >2·0 $\log_{10}$) and a history of roductive problems attributed to CHV-1 as reported by the eder (Table 1).

Sixty-one of the bitches were vaccinated according to the cination schedule and 28 received the placebo. They were included in the study just after mating until the predetermined number of animals in the breeding unit had been reached, and were assigned at random to the vaccinated or the placebo group by using a three-element permutation table. The bitches and their offspring were monitored until the puppies were weaned. The rates of pregnancy, and numbers of stillborn and weaned pups were compared between groups by using a chi-squared test or Fisher's exact test (Statgraphics SGWIN3 software). In breeding unit 5, the weights of the puppies at birth were compared between the groups by using a Kruskall-Wallis test.

TABLE 1: Canine herpesvirus 1 (CHV-1) status of the seven breeding units

Breeding unit	Mean ELISA CHV-1 titre	History of clinical signs attributed to CHV-1	Number of bitches	Number of vaccinates
1	1·4/(1·0)*	No	21	15
2	1·4/(1·0)	Yes	10	7
3	1·0/(0·6)	Yes	2	1
4	1·0/(0·6)	Yes	6	4
5	ND	Yes	20	14
6	0·8/(0·4)	Yes	10	6
7	ND	Yes	20	14

* Observed ELISA titres/(estimated virus neutralisation titre)
ND Not determined

RESULTS

Serology

The two vaccine injections induced a strong seroconversion and CHV-1-neutralising antibody titres were uniformly high (≥1·7 $\log_{10}$) at the time of challenge. The unvaccinated control bitches remained seronegative (≤0·6 $\log_{10}$).

After the pups were challenged, almost all the control bitches seroconverted as a result of the dissemination of the virus. In the vaccinated bitches, the neutralising antibody titres either remained constant or increased slightly.

The puppies alive three weeks after the challenge had titres between <0·2 and 2·3 $\log_{10}$ (Table 2).

Clinical signs

Three pups in litters 1, 2 and 8 died on day 1 of causes unrelated to CHV-1 infection. Mortality related to CHV-1 infection

TABLE 2: Neutralising antibody titres ($\log_{10}$ VN_{50}) in the 12 bitches at different times and in the puppies at 24 days of age

Bitch	Group	1st injection	2nd injection	Challenge (day 3)	Final (day 24)	Puppies (day 24)
1	Control	ND	ND	<0·2	1·0*	0·9
2	Control	<0·2	<0·2	0·5	1·7	2·2; <0·2; 2·2
3	Control	<0·2	<0·2	<0·2	1·8	1·0; 2·3
4	Control	<0·2	<0·2	<0·2	0·4	ND
5	Control	<0·2	<0·2	<0·2	1·5	1·7; 2·2; 1·7; 1·7; 1·7
6	Control	<0·2	<0·2	<0·2	1·0†	ND
7	Vaccinated	0·6	1·2	2·2	2·2	1·2; 1·2; 1·2; 1·1; 1·7‡
8	Vaccinated	<0·2	1·0	1·7	1·7	1·2; 0·7; 1·7; 1·2; 0·9; 2·2
9	Vaccinated	<0·2	0·4	1·7	2·6	1·4; 1·5
10	Vaccinated	<0·2	1·2	1·7	2·8	2·3; 1·7; 2·2
11	Vaccinated	0·6	1·4	1·7	1·7	0·7; 2·2; 0·6; 0·7; 0·4; <0·2; 1·5
12	Vaccinated	<0·2	1·0	1·7	1·7	1·5; 1·2; 1·2; 1·4; 1·2

* Titre 14 days after challenge
† Titre 13 days after challenge
‡ Titre 23 days after challenge
ND Not done

TABLE 3: Results of the challenge with virulent canine herpesvirus 1 (CHV-1) in the individual puppies of the 12 litters

Puppies	Death (days after challenge)	CHV-1 lesions*	Viral isolation	PCR gD	Diagnosis of CHV-1
Control group					
Litter 1					
1	1	–	ND	ND	–
2	9	+	Bacteria	ND	+
3	9	+	Bacteria	ND	+
4	9	+	+	ND	+
5	12	+	Bacteria	ND	+
6	S	–	+	ND	–
Litter 2					
1	1	–	–	ND	–
2	S	+/–	–	+	After effects
3	S	+/–	Bacteria	+	After effects
4	S	+/–	–	+	After effects
Litter 3					
1	12	+	+	+	+
2	9	+	+	+	+
3	11	+	+	+	+
4	S	+/–	–	+	After effects
5	S	+/–	–	–	After effects
Litter 4					
1	8	+	+	+	+
2	9	+	+	+	+
3	9	+	+	+	+
4	14	+	ND	+	+
Litter 5					
1	12	+	+	+	+
2	13	+	+	+	+
3	S	–	–	–	–
4	S	–	–	–	–
5	S	–	–	–	–
6	S	–	–	–	–
7	S	–	–	–	–
Litter 6					
1	6	+	+	+	+
2	8	+	+	+	+
3	8	+	+	+	+
4	10	+	+	+	+
5	11	+	+	+	+
Vaccinated group					
Litter 7					
1 to 5	S	–	–	–	–
Litter 8					
1	1	–	–	–	–
2 to 6	S	–	–	–	–
Litter 9					
1	4	–	–	–	–
2 and 3	S	–	–	–	–
Litter 10					
1	3	–	–	+	– (Suppurative pneumonia)
2	4	–	–	–	– (Suppurative pneumonia)
3	6	–	–	–	– (Suppurative pneumonia)
4	9	–	–	+	– (Suppurative pneumonia)
5 to 7	S	–	–	–	–
Litter 11					
1 to 7	S	–	–	–	–
Litter 12					
1 to 5	S	–	–	–	–

ND Not done, S Survived to 24 days of age
+ Present, – Absent
* + Acute macroscopic lesions, +/– Sub-acute macroscopic lesions

was observed in five of the six challenged control litters (Table 3), with an overall mortality rate of 62 per cent (18/29). The pups died between the 6th and 14th day after challenge, usually after a short period with a nasal discharge, sneezing, moaning, loss of interest in the mother and uncoordinated movements, but sometimes without noticeable clinical signs.

No CHV-1-induced mortality was observed in the vaccinated group (Table 3), and the puppies showed no clinical signs apart from a mild nasal discharge. Four of the puppies in litter 10 died three, four, six and nine days after the challenge, but their death was not due to CHV-1 (see pathology and viral isolation); one of them sneezed occasionally on day 8 and the bitch had a nasal discharge on day 5.

Pathology

In the control group, lesions typical of CHV-1 infection were observed in the puppies which died between the 6th and 14th day after infection (Table 3). The general appearance of the puppies was often normal, but sometimes underweight. The liver had both discoloured and dark red areas and a frail parenchyma. The spleen was enlarged, discoloured and had petechial haemorrhages. The most specific lesions were observed in the kidneys, which had multiple subcapsular haemorrhages, and mottling and discolouration of the cortical parenchyma. The digestive tract commonly had subserosal haemorrhages, and the mesenteric lymph nodes were hypertrophic and haemorrhagic. The lungs had a heterogeneous, wet and vitreous appearance with both reddish and greyish areas and oedema. Haemorrhagic lesions were occasionally observed in the myocardium.

In litter 6 the main lesions were focal necrosis and haemorrhages. Foci of acute necrosis were observed in the liver, spleen, lungs (acute necrotising pneumonia) and kidneys (necrosis of the tubular epithelium). Nuclear eosinophilic inclusion bodies were occasionally visible at the periphery of the necrotic areas in the liver.

In the puppies alive on day 24, no particular lesions were observed except in a few cases. The pups of litter 2 were underweight and small, and pups 2, 3 and 4 had mild subacute interstitial pneumonia; focal lesions of subacute interstitial nephritis were observed in pup 2, and mild lesions of acute pericholangitis were observed in pups 3 and 4. In litter 3, pups 4 and 5 had subacute interstitial pneumonia, and subacute interstitial nephritis, and lesions of the portal areas of the liver (oedema and inflammatory cellular infiltration) were also observed in pup 4. In litter 5, subacute interstitial pneumonia was observed in pups 5 and 6, and in pup 5 the portal areas of the liver were heavily infiltrated with inflammatory cells. All these lesions were attributed to the effects of CHV-1 infection.

In the vaccinated group, no characteristic lesions were observed in the puppies that died (Table 3). The only lesion in the puppy of litter 8 which died on day 4 consisted of dark red lungs. In puppy 1 of litter 9, the abdominal effusion, peritonitis, congestion of the abdominal organs and lungs, frail and fragile spleen and hypertrophy of mesenteric lymph nodes suggested a generalised bacterial infection.

Puppies 1, 2, 3 and 4 of litter 10 had pneumonia with a bacterial infection. Puppy 4 of the same litter had a deep bite wound on the left mandible and slight pulmonary congestion. The puppies euthanased on day 24 had no gross lesions.

Viral isolation

In the control group, CHV-1 was isolated from the kidneys and lungs of all the puppies that died between the 6th and 14th day after infection, except in litter 1, in which bacterial contamination made it impossible to isolate the virus from puppies 2, 3 and 5 (Table 3). The virus could not be isolated from any of the organs of the puppies which survived the challenge.

No CHV-1 could be isolated from the organs of the puppies of the vaccinated group (Table 3). In particular, the samples collected from puppy 1 of litter 8 which was found dead the day after the challenge, from puppy 1 of litter 9 which had lesions suggesting a bacterial septicaemia, and from puppies 1, 2, 3 and 4 of litter 10 which had suppurative lesions of pneumonia, were all negative.

PCR

All the samples from which the virus was isolated were also positive in the PCR (Table 3), except for those in litter 1, which were not tested by PCR. In addition, the samples collected from puppies 2, 3 and 4 of litter 2 and puppy 4 of litter 3 were positive in the PCR, although no virus was isolated.

In the vaccinated group, the samples tested were negative the PCR, except for those collected from puppies 1 and 4 litter 10 (Table 3).

Comparison of control and vaccinated groups

The groups were compared after eliminating the puppies whose death was not due to CHV-1: the puppies of litters 1, 2 and 8 which died on the day after challenge, the puppy of litter 9 which died of bacterial septicaemia, and the puppies of litter 10 which died of bacterial pneumonia. There were 29 puppies left in the control group and 27 puppies in the vaccinated group (Table 4).

No case of CHV-1 disease was detected in the vaccinated group (0/27) whereas 18 puppies died of CHV-1 infection in the control group (18/29). The three puppies of litter 2 in the control group which survived were included among the protected animals, although they had lesions related to the CHV-1 infection and may have shown clinical signs later in life, for example, ocular or neurological disorders.

The efficacy of the vaccine against neonatal CHV-1 infection was highly significant ($P<0.001$).

TABLE 4: Summary of the results of the challenge

Bitch	Group	Dead (puppies/litter)	Pathology (lesions of CHV-1 infection)*	Viral isolation	PCR gD
1	Control	5/6	4/6	2/5†	No data
2	Control	1/4	0/4	0/4	3/3‡
3	Control	3/5	3/5	3/5	4/5
4	Control	4/4	4/4	4/4	4/4
5	Control	2/7	2/7	2/7	2/7
6	Control	5/5	5/5	5/5	5/5
Bitches 1 to 6	Control	20/31	18/31	16/30	18/24
7	Vaccinated	0/5	0/5	0/5	0/5
8	Vaccinated	1/6	0/6	0/6	0/6
9	Vaccinated	1/3	0/3	0/3	0/3
10	Vaccinated	4/7	0/7	0/7	2/7
11	Vaccinated	0/7	0/7	0/7	0/7
12	Vaccinated	0/5	0/5	0/5	0/5
Bitches 7 to 12	Vaccinated	6/33	0/33	0/33	2/33

* After removing puppies that died from causes other than CHV-1 infection, the number of dead puppies/litter are 18/29 in the control group and 0/27 in the vaccinated group
† One puppy was found dead on the day after the challenge; its samples were not tested
‡ One puppy was found dead on the day after the challenge; its samples were not tested
CHV-1 Canine herpesvirus 1

Field trial

The rate of pregnancy tended to be higher ($P=0.11$) in the vaccinated bitches (82 per cent) than in the controls (67·9 per cent). The rate of mortality between birth and weaning was significantly lower ($P<0.01$) in the vaccinated group (11·4 per cent) than in the controls (27·7 per cent). The rates of stillbirth were similar in the vaccinated litters (12·5 per cent) and the controls (15·1 per cent).

The mean (sd) weight of the 22 Yorkshire terrier pups born to vaccinated bitches (123·8 [52·9] g) was significantly higher ($P=0.02$) than that of the 14 pups born to control bitches of the same breed (85 [35·5] g).

DISCUSSION

Because CHV-1 may infect puppies very early in life, vaccination of the bitch is the only option for actively preventing the disease. Passive immunity derived from neutralising antibodies in the colostrum is known to be protective (Huxoll and Hemelt 1970), and neutralising antibody titres as low as 1/4 have been claimed to be protective (Carmichael 1970). In the authors' experience, however, higher titres are needed, suggesting that vaccinating the bitch to induce a high antibody titre at whelping should help to reduce the losses of puppies in breeding units infected with CHV-1.

The virus is a poor immunogen and the antibody titres decrease rapidly (Appel 1987); for optimal protection it is therefore necessary to vaccinate the bitch towards the end of pregnancy, to boost the CHV-1 neutralising antibody response at whelping. The vaccine tested in this study was shown to be safe in the pregnant bitches, because it had no adverse effect on their reproductive performance, particularly the rate of pregnancy, the number of fetuses and the number of weaned pups.

In the laboratory, CHV-1 infection of the control pups caused the total or partial loss of the litters except for litter 2, in which most of the puppies survived the challenge. However, the puppies were small and underweight, and their lesions of interstitial pneumonia combined with a positive PCR on day 21 were probably after effects of CHV-1 infection.

The clinical signs, when they could be detected, were characteristic of CHV-1 infection of puppies, and the mean age of the puppies when they died was in accordance with the incubation period of the disease (four to eight days). The disease was confirmed by specific gross lesions and, in most cases, by the isolation of CHV-1 from the kidneys and lungs.

The vaccination resulted in uniform seroconversion and high neutralising antibody titres (≥ 1.7 $\log_{10}$) in the bitches when the puppies were challenged. There were no cases of CHV-1 disease in the vaccinated group, even in large litters like litter 11 with seven puppies.

The deaths which occurred in the vaccinated group were not related to CHV-1 infection. The only signs of herpesvirus infection were the positive PCR results in two puppies of litter 10 which died of pneumonia. The facts that there were no characteristic lesions, that no CHV-1 could be isolated, and that the disease developed rapidly (puppy 1 died three days after the challenge) suggest that CHV-1 was not responsible for the puppies' death. The presence of viral DNA in the lungs and kidneys of two of these pups may be explained by the slower clearance of CHV-1 virus as a result of a bacterial infection.

The fatal disease of puppies is not the only pathogenic effect of CHV-1. The results of the field trial provided strong evidence of the efficacy of the vaccine against other effects induced by the virus. The higher rate of pregnancy in the vaccinated bitches suggests that CHV-1 may have an adverse effect on fertility. Hashimoto and others (1982, 1983) described the pathogenic role of CHV-1 during pregnancy but there are no data on its role during the early stage of pregnancy. In heifers, bovine herpesvirus-1 has been shown to be responsible for early embryonic death and infertility (Miller and Van Der Maaten 1987). Several mechanisms have been suggested, such as infection of the conceptus, pathological changes in the endometrium, and lesions of the corpus luteum which might decrease its rate of secretion of progesterone. Vaccination in a CHV-1-infected environment favoured the birth of pups of normal size, an effect which has not previously been described. The difference in weight at birth may be related to the pathogenicity of CHV-1 for the placenta resulting in lesions which may be responsible for poor fetal development, leading either to stillbirth or to the birth of undersized pups (Hashimoto and others 1979).

The immunogenicity of the vaccine in field conditions was demonstrated by the uniform seroconversion in the vaccinated population (even in animals with high antibody titres at the first injection); within a week after whelping, the mean ELISA anti-CHV-1 gB antibody titres were 2·3 $\log_{10}$ in the vaccinated group and 1·2 $\log_{10}$ in the control group (data not shown).

As a result of its different pathogenic roles, CHV-1 may cause severe reproductive problems and high economic losses in breeding units. The difficulty in diagnosis (except for the neonatal disease) and the absence of medical or preventive treatment have been responsible for the poor awareness and concern of veterinarians in charge of breeding units. New

techniques, such as PCR, and this vaccine should help veterinarians and breeders to prevent some of the adverse effects of CHV-1.

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SHORT COMMUNICATIONS

Evaluation of imidacloprid for the treatment and prevention of cat flea (*Ctenocephalides felis felis*) infestations on rabbits

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RABBITS are increasing in popularity as house pets in Europe and North America. They are vulnerable to infestation with the cat flea (*Ctenocephalides felis felis*) if kept in an environment contaminated with off-host developmental stages of this parasite. *C felis* is commonly found on both cats and dogs (Rust and Dryden 1997). Eggs laid in the pelage soon fall to the ground and become widely disseminated throughout the home, thereby providing a reservoir of developing eggs, larvae, pupae and newly emerged host-seeking adults (Robinson 1995). Flea populations on pet rabbits are usually modest in number but may be a source of irritation to their host and a concern to humans (Timm 1988).

Few animal health products are specifically licensed for use on rabbits. Caution is needed when extrapolating from recommendations designed for the cat or dog as differences in drug distribution and metabolism may influence efficacy or safety. Also, rabbits obviously differ with regard to skin and hair type. It is therefore of value to have sound scientific data to support the use of parasiticides on this species. Imidacloprid is a highly effective insecticide in widespread use for flea control on cats and dogs. A single topical application of the commercially available 10 per cent spot-on formulation, at a dose rate of 10 mg/kg (the minimum dosage when label recommendations are followed), gives 100 per cent efficacy against a resident *C felis* population and at least 95 per cent protection against reinfestation for four weeks on both cats (Jacobs and others 1997) and dogs (Arther and others 1997). The present study evaluates the use of this formulation on artificially infested laboratory rabbits. The cat dosage schedule was used as cats and rabbits are approximately equivalent in terms of size and surface area: body-weight ratio (Timm 1988).

Six male and six female murex half lop-eared rabbits were caged individually in an environmentally controlled room under conditions complying with animal welfare regulations. Body weights ranged from 2·5 - 3·7 kg. For allocation to treatment and control groups, each rabbit was infested with 100 unfed adult fleas eight days prior to the treatment date (day −8). One day later (day −7), the number of fleas that had established on each was estimated by the thumb-comb technique, described below, and the fleas destroyed. After ranking according to sex and flea-count, matched pairs were formed and the members of each assigned randomly to the two groups. All rabbits were infested with 100 unfed *C felis* RVC1 strain) one day before the treatment date and at weekly intervals thereafter, that is, on days −1, 7, 14, 21 and 28. On day 0, rabbits in the treatment group were each given the contents of one prepackaged 0·4 ml applicator of 10 per cent imidacloprid spot-on formulation (Advantage 40 for Cats; Bayer) as a single topical application onto the skin of the dorsal midline, behind the base of the skull. The rabbits were treated at 30-minute intervals so that flea counts could be started precisely eight hours and 24 hours after treatment. Further