RH: Distemper vaccination of African wild dogs

MODIFIED LIVE DISTEMPER VACCINES CARRY LOW MORTALITY RISK FOR CAPTIVE AFRICAN WILD DOGS, *LYCAON PICTUS*

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30   Abstract: Recently, canine distemper virus (CDV) has been linked to population

declines in the endangered African wild dog (*Lycaon pictus*). As CDV appears able to

persist in wildlife, threats to free-ranging wild dogs cannot be eliminated by vaccinating

domestic dogs. Conservation managers may therefore consider CDV vaccination of wild

dogs in highly threatened populations. For use in field conservation, the ideal CDV

35   vaccine would be safe, immunogenic, and readily available in Africa. The CDV vaccine

type most commonly used for domestic dogs (modified live vaccine) is available in

Africa, and apparently immunogenic in wild dogs, but has been linked to fatal vaccine-

induced distemper in captive wild dogs. However, alternatives are either ineffective

(inactivated vaccine) or difficult to obtain in Africa (recombinant vaccine). Data from a

40   questionnaire survey of zoo vaccination practices were therefore combined with studbook

tracing to assess the safety of modified live CDV vaccine in captive African wild dogs.

Among 135 wild dog pups given modified live CDV vaccine for the first time, there was

a single, unconfirmed, case of potential vaccine-induced distemper. Pups given modified

live vaccine survived better than those given inactivated vaccine or no vaccine. Although

45   studbook tracing revealed higher overall pup survival at zoos which responded to the

questionnaire than at zoos which did not, tracing of all pups born during a 20-year period

which lived long enough to be vaccinated (n=698 pups in 155 litters) revealed no

mortality events consistent with vaccine-induced distemper. Modified live CDV vaccine

thus appears to carry low mortality risks for African wild dog pups in captivity, and may

50   warrant trials in free-ranging populations.

INTRODUCTION

55   The threat to wildlife populations from infectious disease is increasingly recognised[40],

but this recognition seldom prompts intervention in the wild.[52,63] In mammals, the

pathogens which threaten wildlife populations are often shared with domestic animals,

yet vaccines which are safe and effective in domestic animals may be ineffective or even

harmful in wildlife.[8,54,63] Hence, concerns about vaccine safety have impeded the

60   development of field vaccination programmes for several wildlife species.[30,6]

      The African wild dog, *Lycaon pictus*, is an endangered species which is threatened by

infectious disease. Rabies has been considered the greatest disease threat, since it was

confirmed in the Serengeti wild dog population just before that population went extinct,

and has also thwarted reintroduction attempts in Namibia and South Africa.[18,27,65,49,23]

65   Canine distemper virus (CDV) was previously thought to be a less serious threat than

rabies, because seropositive wild dogs were reported from many healthy populations,

suggesting widespread nonlethal exposure.[13,41,31,5,2] Moreover, prior to 2016, the only

major CDV outbreak confirmed in wild dogs had occurred in captivity, where packs were

held in close proximity; the only two confirmed outbreaks in wild populations were

70   restricted to single packs.[1,20,57] However, in 2016-7 CDV killed ≥20 packs in Kenya's

Ewaso ecosystem, three packs in South Africa, and one pack in Tanzania.[15,21,32,38] There

is evidence, therefore, that CDV can represent a serious threat to free-ranging wild dog

populations. This pattern of highly variable mortality, with widespread nonlethal

exposure and occasional mass mortality, is also a feature of CDV epidemiology in some

75   other carnivore species.[47,37,56,10]

Strategic plans for wild dog conservation call for mitigation of disease threats.[24,26,25] For rabies, mass vaccination of domestic dogs is likely to reduce these threats, as wild dogs acquire rabies mainly from domestic dogs and, fortuitously, ongoing strategies to end human rabies deaths entail domestic dog vaccination on the very large scales needed

80 to protect wild dogs.[42,64,66,9,45,67] In contrast, domestic dog vaccination may contribute little to protecting wild dog populations from CDV. The virus appears not to persist in domestic dog populations at the local scale, with several studies suggesting persistence in wildlife.[42,19,10] Wild dog exposure to CDV is not associated with domestic dog contact, and mass CDV vaccination of domestic dogs in the Serengeti ecosystem failed to prevent

85 new wildlife infections.[61,41,66] Where there is a recognised need to protect free-ranging wild dogs from CDV, conservation managers may therefore need to consider vaccination of wild dogs themselves.

For use in the wild, the ideal CDV vaccine would be safe, immunogenic, and readily available in African wild dog range states. Unfortunately, it is not clear that any of the

90 three types of CDV vaccine (modified live, inactivated, and recombinant) meet all three criteria. Modified live CDV vaccine is widely used in domestic dogs, including in Africa; however it has been reported to cause fatal vaccine-induced distemper in African wild dogs, as well as some other carnivore species.[8,35,58,16,22] Inactivated vaccine carries no such risk of vaccine-induced distemper; however it has not prompted strong immune

95 responses and failed to prevent mass mortality at a wild dog captive breeding facility in Tanzania.[36,62,57] Recombinant CDV vaccine has been shown to trigger immune responses in captive wild dogs, but supply issues can limit access to this vaccine type, and

restrictions on the import of genetically modified organisms to many African countries could hinder its use in wild populations.[7,29,11]

100   For North American zoos, husbandry guidelines recommend the use of recombinant CDV vaccines, to avoid any risk of vaccine-induced distemper.[4] In Europe, however, recombinant CDV vaccines are not available and modified live vaccine is widely used. Limited studies suggest that modified live CDV vaccine is immunogenic in captive wild dogs.[59,51] A recent study of captive tigers (*Panthera tigris*) found that

105   modified live CDV vaccines were markedly more immunogenic than recombinant vaccines.[48]

The immunogenicity and widespread availability of modified live CDV vaccines mean that this vaccine type would be the best tool for field conservation, if the risks of vaccine-induced distemper were acceptably low. Although several cases of vaccine-

110   induced distemper have been reported in African wild dogs, cases when modified live distemper vaccine caused no ill-effects are seldom reported, and so the risk of vaccine-induced distemper is uncertain. Moreover, risks may have changed over time in response to changing conditions. A widely-used vaccine strain (the Rockborn strain, adapted to canine kidney cell culture) which was controversially linked to vaccine-induced

115   distemper was withdrawn during the 1990s, potentially reducing the risk in subsequent years.[33] Additionally, there is some evidence that the immunosuppressive effect of parvovirus infection may increase risks of vaccine-induced distemper, and so the extent of parvovirus control may influence the safety of modified live CDV vaccine.[28] Finally, the use of multivalent preparations including modified live vaccines against both

120   distemper and parvovirus has been tentatively linked to the risk of vaccine-induced

distemper.[53]

This study therefore evaluated the mortality risks associated with administering

modified live CDV vaccine to African wild dogs in captivity, by combining studbook

data with data from a questionnaire survey of zoos, conducted in the years before

125   recombinant CDV vaccines became available.

MATERIALS AND METHODS

Published reports

130   Published accounts were used to characterise the mortality patterns associated with

vaccine-induced distemper, including timing of clinical signs and death relative to

vaccination, and mortality rate within affected litters.[35,58,16]

Questionnaire survey

135   Questionnaire data were gathered in 2000, by sending paper forms to veterinary staff

responsible for the care of wild dogs held in captivity. Questionnaires were sent to 55

collections worldwide, listed on the International Species Information System (ISIS) as

holding wild dogs within their collections at the time, and to two unlisted private

collections known to hold wild dogs. The questionnaire asked for data on current and past

140   policy on vaccinating wild dogs (with reasons for any changes), and whether any wild

dogs had ever become sick or died following the administration of vaccines. In addition,

data were requested on the vaccination histories (vaccination dates, vaccine brand names

and, where possible, batch numbers) of any wild dogs held. Data were not requested on incidents of wild-type distemper. To maximise responses, the questionnaire was worded

145 to reassure participants that information from individual collections would be held in confidence. Questionnaires were sent a second time to collections that had not responded 4 months after the initial mailing.

Where zoos provided individual vaccination records, an independent estimate of mortality post-vaccination was obtained by using studbooks (which cover the European

150 and North American captive populations) to trace the survival of vaccinated animals.[46,60] As questionnaires were sent worldwide, not just to the regions covered by the studbooks, the fates of some animals mentioned in the questionnaire responses could not be traced in this way. A Cox proportional hazards model (including litter identity as a random effect, fitted using package *coxme* in the statistical package *R*) was used to compare the survival

155 of pups which were, and were not, given modified live CDV vaccine.[55,44] The Cox proportional hazards model is a type of survival analysis, which allows assessment of associations between independent variables (e.g., vaccination) and the time taken for an event to occur (e.g., death).[12] A hazard ratio less than one indicates a lower probability of death (i.e., higher survival). The inclusion of a random effect (here, litter identity)

160 accounts for the statistical non-independence of pups from the same litter, as they are genetically related, exposed to the same environmental conditions, and likely to receive vaccine from the same batch.

Studbook analyses

165　　Questionnaire surveys will give inaccurate results if respondents represent a biased

sample of the population. To assess whether the zoos which returned their questionnaires

were representative, studbook data were used to estimate pup survival across the

European and North American captive populations during the period covered by most

questionnaire responses (1980-2000). Pup survival was compared between zoos which

170　　did, and did not, respond to the questionnaire survey using a Cox proportional hazards

model including litter identity as a random effect, fitted using package *coxme* in the

statistical package *R*.[55,44]

175　　　　　　　　　　　　　　　　　　　RESULTS

Published reports

　　All of the published reports of vaccine-induced distemper involved pups given their

first vaccinations (Table 1). All pups had been given modified live CDV vaccine in a

polyvalent formulation which also included parvovirus vaccine; however, some

180　　formulations included modified live parvovirus vaccine while others contained

inactivated parvovirus vaccine (Table 1).

　　Clinical signs were observed 14.2 days post-vaccination on average (SD 5.6 days),

with death occurring 17.8 days (SD 6.2 days) post-vaccination (Table 1). Across the four

affected litters, 20 of 21 pups died (Table 1; 95.2% mortality, exact binomial 95%

185　　confidence interval (CI) 76.2-99.9%), with deaths occurring across a period of 2-11 days

within each litter (Table 1).

Questionnaire responses

190   Questionnaires were returned by 36 collections (a 63% response rate), of which 26

reported having vaccinated wild dogs against CDV. Of 13 zoos which gave details of

vaccines used, 12 had used modified live CDV vaccines, in either monovalent (Fervac

[United Vaccines, Fitchburg WI 53593, USA], Galaxy-D [Schering Plough, Omaha, NE

68138, USA], Fromm-D [Solvay, Mendota Heights, MN 55120, USA]), or multivalent

195   (Candur [Behringwerke, Marburg 35041 Germany], Canvac [Dyntec, Terezin 41155,

Czech Republic], Duramune [Elanco, Fort Dodge, IA 50501, USA], Kavak [Fort Dodge,

Overland Park, KS 66210, USA], Nobivac [Intervet, Millsboro, DE 19966, USA],

Protech [Arthur Webster Pty, Sydney, Australia], Vanguard [Pfizer, Ramsgate CT13

9ND, UK]) preparations. Two captive facilities had used both inactivated and modified

200   live CDV vaccines, and one had used inactivated vaccine exclusively (CDV-ISCOM,

Rotterdam 3720, Netherlands).

One hundred and thirty-five wild dogs were reported to have received modified live

CDV vaccine for the first time at known age, although the vaccine brand was only

reported for 37 of these animals. These first vaccinations were given at a mean age of 61

205   days (SD 43 days, range 40-217 days), with 90% of animals having received their first

vaccination by the age of 67 days (Figure 1A).

Of 26 zoos which reported having vaccinated wild dogs against CDV, 25 reported no

observations of ill-effects post-vaccination. One zoo reported a case of a single wild dog

pup which died, aged 82 days, 10 days after its second dose of modified-live CDV

210   vaccine. Neither the brand nor type of vaccine (monovalent/polyvalent) was reported.

The *post mortem* report provided a diagnosis of canine distemper based on clinical symptoms, necropsy, and histopathology, and CDV was confirmed by a fluorescent antibody test. However, failure to isolate the virus in cell culture led the virologist consulted to suggest that this was likely to have been a wild-type virus, since "...*vaccine strains of CDV are highly cell-adapted and usually will propagate in cell cultures...*" (Unpublished necropsy report). This animal had seven littermates; one died aged 43 days, on the day of first vaccination (and so could not have died of vaccine-induced distemper), and another died aged 57 days, following bite-wounding. Five other littermates survived to adulthood. This incident therefore entailed lower post-vaccination mortality than the published cases shown in Table 1 (28.6% *vs* 95.2% mortality; Fisher exact P = 0.001). Nevertheless, if the two animals which died post-vaccination are assumed to have contracted vaccine-induced distemper, it would suggest a maximum of two deaths among 135 vaccinated animals, giving a vaccine-associated mortality estimate of 1.5% (CI 0.2-5.3%). If this incident did not represent vaccine-induced distemper, the estimate would be 0% (CI 0-2.7%).

Studbook tracing revealed that 118 pups given a modified live CDV vaccine in Europe or North America (aged 44 days on average) experienced higher survival over the subsequent 180 days than 17 pups (aged 44 days) given either no CDV vaccine (n=14) or inactivated CDV vaccine (n=3; Cox proportional hazards model including litter identity as a random effect, effect of modified live vaccine: hazard ratio = 0.142, P = 0.012, Figure 1B). The survival of pups reported to have been given modified live distemper vaccine in a monovalent formulation (n=15) could not be compared with that of pups given polyvalent formulations containing parvovirus vaccine which was either inactivated

(n=6) or modified live (n=15), because none of these 36 animals died in the 180 days

235   following vaccination.

Studbook analyses

        Studbooks recorded mortality at all ages: of 1,459 pups born in the years 1980-2000,

40% died in the first five days of life, and 42% survived more than six months (Figure

240   1C).

        The minimum age for vaccine-induced distemper (45 days) was estimated by adding

the minimum reported age at first vaccination (40 days; Figure 1A) to a conservative

estimate of the time from vaccination to death, based on published studies (5 days,

calculated as the mean [17.8 days] minus 2SD [12.4 days]; Table 1). The likely maximum

245   age for vaccine-induced distemper (99 days) was calculated by adding the age by which

90% of pups had received their first vaccination (67 days; Figure 1A) to the maximum

reported time from vaccination to death (32 days; Table 1). Among zoos which were sent

questionnaires, pup survival at ages 45-99 days was consistently higher at zoos which

responded to the questionnaire than at those which did not respond (Cox proportional

250   hazards model including litter identity as a random effect, effect of zoo response: hazard

ratio = 0.322, P = 0.045, Figure 1D).

        As survival was consistently higher at zoos which responded to the questionnaire, an

alternative estimate of potentially vaccine-induced mortality was derived, independent of

the questionnaire results. Litters were identified as potential cases of vaccine-induced

255   distemper if more than one pup died at age 45-99 days, since published accounts reported

mortality of over 75% in affected litters (Table 1). To help interpret mortality patterns,

data were also collated on deaths aged 20-44 days and 100-175 days in these litters (Table 2).

Among 155 litters with members surviving to 45 days (totalling 698 pups), 18 litters
260   met this criterion, with 53 pups dying aged 45-99 days (Table 2). If all of these litters had received modified live CDV vaccine, and all of these deaths had reflected vaccine-induced distemper, the vaccine-associated mortality would be 7.6% (CI 5.7-9.8%). However, these 18 incidents represented marked lower mortality within affected litters than the published cases (42.4% vs 95.2%, Fisher exact $P < 0.001$). The only case of
265   100% mortality aged 45-99 days involved a litter from a zoo which had reported that it did not routinely vaccinate wild dog pups against CDV (Table 2). Moreover, in 10 of the 18 litters, the reported deaths were part of a series which extended before or after the period when pups were 45-99 days old (Table 2). One additional litter (which experienced no mortality aged 45-99 days, and is therefore not shown in Table 2)
270   experienced 100% mortality (of three pups) aged 100-175 days, but this litter was confirmed to have received inactivated CDV vaccine, not modified live vaccine. With no litters showing the brief episodes of high mortality reported in the published cases, at the age when vaccine-induced distemper would be expected, the incidence of apparent vaccine-associated mortality in pups aged ≥45 days appears to be 0/698 (0%, CI 0-
275   0.53%). However, it is not known how many of the 698 pups had in fact received modified live CDV vaccine.

DISCUSSION

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The results presented here suggest that modified live distemper vaccine carries a low mortality risk for captive African wild dogs. Zoos reported only one potential case of vaccine-induced distemper among 135 pups given modified live distemper vaccine, and this case was not confirmed, differing in several ways from published accounts. Although

285 the zoos which responded to the questionnaire represented a biased sample of wild dog litters with relatively low mortality, an independent evaluation of studbook records revealed no patterns of mortality similar to those described in published accounts of vaccine-induced distemper. Indeed, studbook tracing revealed that wild dog pups which received a modified live distemper vaccine experienced significantly lower mortality than

290 those which did not.

The difference in pup mortality recorded at zoos which did, and did not, respond to the questionnaire represents a form of non-response bias, a well-known source of bias in postal surveys.[50] Non-response bias was evaluated because zoo staff might have been reluctant to report cases of vaccine-induced distemper, and such reluctance is one

295 potential explanation for the higher pup mortality observed at non-responding zoos (Figure 1D). However, this explanation is not consistent with analyses of studbook data, which did not reveal mortality patterns similar to published cases of vaccine-induced distemper, in either responding or non-responding zoos. An alternative explanation is that non-responding zoos may not have routinely vaccinated wild dog pups against CDV, a

300 practice which was also associated with lower pup survival (Figure 1B). Reassuringly, the questionnaire survey and the studbook analysis both suggested consistently low risks of vaccine-induced distemper.

Wild dog pups given either no CDV vaccine or inactivated vaccine experienced lower survival than those given modified-lived CDV vaccine (Figure 1B). Potentially, this

305   pattern might reflect mortality from wild-type CDV, which has been reported from some captive facilities, including one where wild dogs had been given inactivated CDV vaccine.[3,39,57] However, this pattern might also have reflected the protection against other canine pathogens afforded by the multivalent vaccines that most zoos used. Additionally, routine pup vaccination could indicate greater attention to veterinary care, improving

310   survival. Irrespective of the mechanism behind it, this finding showed that modified live CDV vaccine was not associated with elevated pup mortality.

This study revealed a single case of confirmed distemper in a vaccinated wild dog pup, suspected by the virologist to reflect infection with wild-type virus rather than a vaccine strain. This observation is consistent with a study of domestic dogs, in which

315   molecular analyses revealed that 10 puppies with suspected vaccine-induced distemper were instead infected with wild-type CDV.[34] This finding may also cast doubt on the published cases of vaccine-induced distemper, none of which could rule out the possibility of wild-type CDV infection.[35,58,16]

It is surprising that four cases of presumed vaccine-induced distemper were published

320   during the 1980s (Table 1), when similar mortality events were not recorded in studbooks during 1980-2000 (Table 2). Three of the published cases involved litters born outside the captive populations monitored by the studbooks, and the fourth case (reported from an unspecified zoo on an unspecified date) could not be identified within studbooks. The literature on CDV vaccines' reversion to virulence suggests several possible explanations

325   for these potential cases of vaccine-induced distemper. Vaccine strain variation is one

explanation; however, the four published cases of presumed vaccine-induced distemper involved at least two different vaccine strains, including the Onderstepoort strain which is widely considered safe (Table 1).[14,33] Likewise, immunosuppression caused by coinfection with parvovirus (or even modified live parvovirus vaccine) has been proposed

330   as a cause of vaccine-induced distemper,[17,28] but only two of the four reported cases involved modified live parvovirus vaccine (Table 1). Alternatively, host genetic susceptibility has been linked to vaccine-induced distemper in domestic dogs, and it is possible that the careful genetic management of captive populations (which developed from the 1980s onwards, and is the purpose of maintaining studbooks) might have

335   reduced the risk of vaccine-induced distemper.[17,43]

Evidence concerning the safety of vaccines in captive animals is, of course, only one component of the information needed to evaluate vaccination as a conservation tool for free-ranging animals. Evidence would also be needed of vaccine safety and effectiveness in the wild, where animals are more likely to be nutritionally stressed, as well as

340   immunologically challenged by other pathogens. The apparently low mortality risk associated with modified live CDV vaccines in captive African wild dogs, combined with the apparently high immunogenicity of such vaccines, and their widespread availability in Africa, suggest that conservation managers may wish to consider a field trial to evaluate them further.

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550   Table 1. Characteristics of presumed vaccine-induced distemper in African wild dogs as reported in the literature. All published cases involved pups being given their first dose of modified-live distemper vaccine, in a polyvalent formulation including canine parvovirus (CPV) vaccine. "NR" indicates data information which was not reported.

Litter	Individual	Year	Vaccine type	Age at vaccination (days)	Days to clinical signs	Days to death	Ref
A	A1	<1989	Candur SHLP,	57	18	22	16
A	A2	<1989	Behringwerke,	57	24	30	16
A	A3	<1989	Marburg 35041.	57	25	31	16
A	A4	<1989	Strain: Rockborn CPV: inactivated	57	26	32	16
B	B1	1981		150	10	12	35
B	B2	1981	Paramune 5, Dellen	150	10	13	35
B	B3	1981	Laboratories, Omaha,	150	10	15	35
B	B4	1981	NE 68134, USA.	150	10	17	35
B	B5	1981	Strain: unknown	150	10	19	35
B	B6	1981	CPV: inactivated	150	10	20	35
B	B7	1981		150	10	survived	35
C	C1	<1987	Quantum 4, Pitman-	56	13	15	58
C	C2	<1987	Moore, Washington	56	13	15	58
C	C3	<1987	Crossing NJ 08560,	56	13	15	58
C	C4	<1987	USA.	56	13	16	58
C	C5	<1987	Strain: Onderstepoort	56	13	16	58
C	C6	<1987	CPV: live	56	13	15	58
D	D1	<1987	Quantum 6, Pitman-	77	NR	13	58
D	D2	<1987	Moore, Washington Crossing NJ 08560,	77	NR	13	58
D	D3	<1987	USA.	77	NR	13	58
D	D4	<1987	Strain: Onderstepoort CPV: live	77	NR	14	58
	mean ±SD			91.5 ±43.1	14.2 ±5.6	17.8 ±6.2	

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Table 2. Captive African wild dog litters identified through studbook tracing to have experienced ≥2 deaths aged 45-99 days (when the risk of vaccine-induced distemper was estimated to be highest). Deaths before and after this age are also shown; "na" (not applicable) indicates that no deaths were recorded within the stated time window. CDV

560 vaccination status is reported as "unknown" if zoos were not sent questionnaires, or did not return them, as "yes"/ "no" if zoos provided vaccination records for these individuals, and "probably"/ "probably not" if zoos only indicated their typical vaccination practices.

Zoo	Litter	Pups alive at 45 days	Mortality 45-99 days	Days of age at death			CDV vaccinated?
				20-44	*45-99*	*100-175*	
A	E284	5	40%	na	65,65	na	unknown
A	E354	4	50%	na	79,87	105,114	unknown
B	E286	8	25%	na	77,84	na	unknown
B	E287	10	30%	29	56,56,72	na	unknown
B	E288	5	80%	39	48,65,65,77	na	unknown
B	E289	8	50%	35	45,54,88,91	na	unknown
C	N51	8	25%	na	56,56	na[a]	unknown
D	E166	8	63%	na	79,81,82,82,92	na	unknown
E	E197	9	22%	na	50,50	na	unknown
F	E257	3	67%	35	53,70	na	unknown
F	E260	10	20%	36	65,68	127,155	unknown
G	E92	8	100%	39	49,53,53,56,58,63,66,66	na	probably not
G	E95	8	25%	29,41	53,55	na	no
H	E233	6	50%	na	85,86,91	na	probably not
I	E390	9	33%	na	62,74,80	na	probably
J	N6	7	28%	43	57,82	na	yes - live
K	N46	6	50%	na	50,54,55	na	unknown
L	E266	3	67%	33,40	62,71	na	unknown
	Total	125	42.4%				

[a]three littermates transferred and lost to monitoring at 101 days of age

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Figure 1 Vaccination of captive African wild dogs against Canine Distemper Virus (CDV). Panel A shows age at first vaccination for 135 pups given modified live CDV vaccine; the dotted line indicates the age by which 90% of pups had been vaccinated. Panel B compares the survival of pups first given modified live CDV vaccine at known

570 age in North America and Europe, with those given either no vaccine or an inactivated vaccine. Panel C shows the survival, from birth to 180 days, of 1,459 pups born in North America and Europe in 1980-2000, with shading showing the age (45-99 days) when the risk of vaccine-induced distemper was estimated to be highest. Panel D compares the survival, from 45-99 days, of pups born at zoos which did, and did not, respond to the

575 questionnaire about vaccination practices.