Effective vaccination against rabies in puppies in rabies endemic regions

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Abstract

In rabies endemic regions, a proportionally higher incidence of rabies is often reported in dogs younger than 12 months of age, which includes puppies less than 3 months of age; this presents a serious risk to public health. The higher incidence of rabies in young dogs may be the effect of low vaccination coverage in this age class, partly as a result of the perception that immature immune systems and maternal antibodies inhibit seroconversion to rabies vaccine in puppies less than 3

months of age. Therefore, to test this perception, we report the virus neutralizing antibody titres from 27 dogs that were vaccinated with high quality, inactivated rabies vaccine aged 3 months of age and under as part of larger serological studies undertaken in Gauteng Province, South Africa, and the Serengeti District, Tanzania. All of these dogs seroconverted to a single dose of vaccine with no adverse reactions reported and with post-vaccinal peak titres ranging from 2.0 – 90.5 IU/ml. In light of these results, and the risk of humans contracting rabies from close contact with puppies, we recommend that all dogs in rabies endemic regions, including those less than 3 months of age, are vaccinated with high quality, inactivated vaccine.

Introduction

Canine-mediated human rabies kills approximately 60,000 people every year (WHO 2013). Mortality from rabies is highest in less developed communities in Asia and Africa where domestic dogs are free-roaming (Butler and Bingham 2000; Ezeokoli and Umoh 1987; Kasempimolporn and others 2008; Kayali and others 2003; Kitala and others 2002; Reece and Chawla 2006; WHO 2013; Windiyaningsih and others 2004); with increasing evidence that the majority are owned (Butler and Bingham 2000; Estrada and others 2001; Ezeokoli and Umoh 1987; Gsell and others 2012; Morters and others 2014b; van Sittert and others 2010; Windiyaningsih and others 2004) and, thus, generally accessible for vaccination (Knobel and others 2013; Lembo and others 2010).

Mass vaccination of domestic dogs is key to the successful control of canine rabies, and a strong body of theoretical and empirical evidence indicates that vaccinating 70% of the dog population during annual campaigns should be sufficient to control rabies (Belotto and others 2005; Cleaveland and others 2006; Cleaveland and others 2003; Coleman and Dye 1996; Hampson and others 2009; Schneider and others 2005; WHO 2013). Achieving vaccination coverage of 70% during campaigns should maintain population immunity above the critical levels of 20-45% required to interrupt rabies

53 transmission (Coleman and Dye 1996; Hampson and others 2009). Effective coverage has been
54 achieved through vaccinating juveniles and adults (Beran 1991; Chomel and others 1987; de Balogh
55 and others 1993; Flores-Ibarra and Estrella-Valenzuela 2004; Matter and others 2000; Mitmoonpitak
56 and others 1998; Touihri and others 2011), given that puppies less than 3 months of age are often
57 excluded from vaccination programs (Awoyomi and others 2007; Beran and Frith 1988; Brooks 1990;
58 Chomel and others 1987; Durr and others 2009; Flores-Ibarra and Estrella-Valenzuela 2004;
59 Gunatilake and others 2003; Matter and Fico 1998; Matter and others 2000; Mitmoonpitak and
60 others 1998; Touihri and others 2011).
61
62 Low vaccination coverage in puppies has important implications for public health, especially as
63 vaccination coverage of the population and, thus, herd immunity declines following a vaccination
64 campaign. A proportionally higher incidence of rabies is often reported in dogs under 12 months of
65 age, which includes puppies less than 3 months of age (Belcher and others 1976; Beran 1991;
66 Malaga and others 1979; Mitmoonpitak and others 1998; Widdowson and others 2002). In these
67 studies, the proportion of laboratory confirmed cases in dogs under 3 months of age range from
68 7.6% to 17.4%. This presents a serious risk to the public, given that the fraction of puppies less than
69 3 months of age in a population may be large, reportedly ranging from 4.1% to 39% (Davlin and
70 VonVille 2012), and the close relationship between humans and puppies (Awoyomi and others 2007;
71 Mitmoonpitak and others 1997; Taiwo and others 1998; WHO 1998; Widdowson and others 2002).
72
73 Puppies less than 3 months of age are generally excluded from rabies vaccination programs on the
74 assumption that they have immature immune systems and maternal antibodies (Day 2007; Hodgins
75 and Shewen 2012; Siegrist 2008) which may limit the immune response to rabies vaccine. Primarily
76 to safeguard against possible inhibitory effects of maternal antibody, most manufacturers of high
77 quality, inactivated rabies vaccines for dogs recommend a primary or booster vaccination at 12-13
78 weeks (Merial Animal Health Limited ; MSD Animal Health). Similarly, internationally recognised

vaccination guidelines for dogs recommend primary vaccination against rabies at 12-13 weeks of age (AAHA 2011; WSAVA 2010). Consequently, those administering vaccine under field conditions may be reluctant to use rabies vaccines off-label (Awoyomi and others 2007; Touihri and others 2011); even though World Health Organization (WHO) guidelines recommend that all dogs, including puppies less than 3 months of age, are vaccinated during mass vaccination campaigns (WHO 2004, 2013) when booster vaccinations are generally not available. Furthermore, owners also often perceive puppies as too young for vaccination (Davlin and others 2013; Flores-Ibarra and Estrella-Valenzuela 2004; Kaare and others 2009; Kongkaew and others 2004) so they are often not presented for vaccination during campaigns.

Evaluation of the effect of maternal antibodies and immune function of puppies on rabies vaccine induced immune responses is limited. Maternal antibody may interfere with immune responses (Day and Schultz 2011; Siegrist 2012; Tizard 2013), particularly in puppies 8 weeks of age or younger vaccinated with modified live rabies vaccine under field conditions (Aghomo and others 1990). However, at least under experimental conditions, maternal antibodies and immune function may not limit the immune response to inactivated vaccines which stimulate both B- and T- cell responses (Siegrist 2012), as demonstrated in puppies vaccinated with Rabisin (Merial Animal Health Limited) at 2 weeks of age (Chappius 1998). We present serological data from puppies vaccinated under field conditions in South Africa and Tanzania that support these prior observations.

Materials and methods

Puppies (hereafter defined as dogs 3 months of age and under) were vaccinated as part of larger serological studies in five low-income communities of Africa where the dogs are owned, with the majority being mixed-breed and free to roam. The five communities include the township of Zenzele in Gauteng Province, South Africa (Morters and others 2014a; Morters and others 2014b), and four

105 villages (Ngarawani, Runga'bure, Nyamburi and Bisarara, hereafter referred to as "Serengeti") in the
106 Serengeti District, Tanzania (McNabb 2008). Vaccinations for this study were undertaken during
107 February 2010 in Zenzele and May 2008 in the Serengeti. None of the puppies were vaccinated prior
108 to this study. Central point vaccination campaigns had also been undertaken in Zenzele by the
109 Department of Agriculture (DoA) in May 2006. In the Serengeti, annual central point vaccinations
110 have been undertaken since 2003 as part of studies to investigate and prevent canid diseases (Kaare
111 and others 2009).
112
113 For the puppies in the Serengeti and those acquired from outside of Zenzele, age was reported by
114 the owner but validated by direct observation and tooth eruption (Dyce and others 1987). For
115 puppies born in Zenzele, age was determined from intensive monitoring of the dams generating
116 reliable whelping dates, direct observation and tooth eruption (Morters and others 2014b).
117
118 In Zenzele, every available dog (n=259) in the entire population (of 315), including 68 puppies (from
119 a total of 86 in the population) and their dams, were vaccinated door-to-door with 1ml of Rabisin
120 (Merial Animal Health Limited), an inactivated rabies vaccine containing at least 1 IU of rabies virus
121 glycoprotein GS57 Wistar strain. Vaccine was administered subcutaneously into the nape of the
122 neck.
123
124 In the Serengeti, eight puppies in a convenience sample of 200 dogs brought to a central vaccination
125 station were vaccinated with 1ml of Nobivac Rabies (MSD Animal Health) subcutaneously into the
126 nape of the neck. Nobivac Rabies contains >2 IU inactivated Rabies Virus strain Pasteur RIV. In
127 addition, $1/10^{th}$ of the stated dose of Nobivac Puppy DP, containing live attenuated strains of Canine
128 Parvo Virus (CPV) (strain C154) and Canine Distemper Virus (CDV) (strain Onderstepoort),
129 reconstituted using Nobivac Rabies Virus (MSD Animal Health), was administered to all puppies, and

130 0.01ml/kg of ivermectin (Ivomec) to a proportion of the puppies. In both locations the vaccine cold
131 chain was carefully maintained.
132
133 The majority of the (68) puppies in Zenzele were <6-8 weeks of age when vaccinated and deemed
134 too small to blood sample immediately prior to vaccination without causing un-necessary distress to
135 the puppy and/or owner (see Table S1 for the age distribution at vaccination of the (68) puppies).
136 Therefore, pre-vaccinal virus neutralising antibody (VNA) titres (hereafter referred to as "titres")
137 were obtained from only four of the puppies. To measure post-vaccinal peak titres blood samples
138 were collected approximately 30 days following vaccination. Thirty seven of the 68 vaccinated
139 puppies remained in Zenzele 30 days after vaccination, and of these nineteen were big enough to
140 blood sample (see Table S1 for the outcomes of the (68) vaccinated puppies). In the Serengeti, blood
141 samples were collected from all eight puppies immediately prior to vaccination and 21 days later. All
142 samples were centrifuged within 8 hours of collection, and the sera were either chilled or frozen
143 from the time of collection until they were shipped to the Animal and Plant Health Agency, UK,
144 where titres were measured by fluorescent antibody virus neutralization (FAVN) test, a method
145 prescribed by the Office International des Epizooties (OIE) (Cliquet and others 1998). Aliquots of the
146 sera were also transported chilled from the Serengeti to Cornell University, USA, where titres for
147 CDV and CPV were measured by virus neutralization and haemagglutination inhibition tests
148 respectively.
149
150 All puppies were examined by a veterinarian at the time of vaccination and blood sampling. In
151 Zenzele, every owner was made aware of the emergency phone number (written on their dog's
152 vaccination certificate) to contact the veterinarian if any abnormalities in the health or behaviour of
153 their dog were observed following vaccination. Every house in Zenzele in which a puppy was
154 vaccinated in February 2010 was revisited twice by the veterinarian during March 2010 to collect (i)
155 demographic data by direct observation and owner questionnaire, and (ii) day 30 post-vaccinal

blood samples as part of larger dog demography (Morters and others 2014b) and aforementioned serological (Morters and others 2014a) studies respectively. During each visit, every available vaccinated puppy underwent a health assessment irrespective of whether a blood sample was collected or not. See Table S1 for a description of the health assessments of the (68) puppies vaccinated in Zenzele in February 2010.

The study in South Africa was approved by the Ethics Committee, University of Cambridge, and the Research and Animal Ethics Committees, University of Pretoria, and the study in Tanzania approved by the Tanzanian Commission for Science and Technology, Tanzania Wildlife Research Institute, and the Royal (Dick) School of Veterinary Studies, Edinburgh. In Tanzania the blood samples were collected during an ongoing vaccination program undertaken by the Serengeti Health Initiative. In all cases, vaccination and blood sampling were only carried out with the owner, or responsible adult delegated by the owner, present and their informed consent.

Results

In Zenzele, titres for the four puppies sampled immediately prior to vaccination were ≤0.13 IU/ml, similar to pre-vaccinal titres in 32 dogs 1.5-4.5 months of age from dams vaccinated with high quality, inactivated vaccine against rabies in Thailand (Kasempimolporn and others 1996; Tepsumethanon pers. comm. 2015). Pre-vaccinal titres for the eight puppies in the Serengeti were <0.3 IU/ml (ranging from 0.07 IU/ml to 0.29 IU/ml). Post-vaccinal peak (i.e. day 30) titres for the (19) puppies in Zenzele are shown in Table 1. All of the puppies seroconverted to the vaccine (i.e. generated titres ≥0.5 IU/ml (Kennedy 1998)), with a geometric mean titre (GMT) of 20.7 IU/ml.

Table 1 Day 0 (pre-vaccination) and day 30 (peak) titres of the puppies vaccinated in Zenzele

dog	gender	age at vaccination (weeks)	puppy day 0 titres (IU/ml)	puppy day 30 titres (IU/ml)	dam day 0 titres (IU/ml)	dam present May-06
1	f	8-10	0.06	11.3	0.06	yes
2	m	8-10	0.06	2.0	0.06	yes
3	f	7-8	–	45.3	0.18	yes
4	m	6-7	–	22.6	0.06	no
5	m	6-7	–	45.3	0.06	no
6	f	7-8	–	16.0	0.06	no
7	m	5-6	–	64.0	0.06	no
8	f	4-6	–	45.3	0.06	yes
9	f	5-7	–	32.0	0.06	yes
10	f	5-7	–	64.0	0.06	yes
11	m	5-7	–	5.7	0.06	yes
12	m	4-6	–	45.3	0.18	yes
13	f	4-6	–	90.5	0.18	yes
14	f	5	–	22.6	0.06	yes
15	f	5	–	8.0	0.06	yes
16	f	5	–	5.7	0.06	yes
17	f	10 days	–	5.7	0.09	no
18	f	6-8	–	32.0	–	–
19	f	10-12	–	22.6	–	–

Seventeen of the nineteen puppies (blood sampled on day 30) were born in Zenzele to eight dams; all of the adult females were seronegative (<0.5 IU/ml) immediately prior to vaccination in February 2010 (Table 1). Five of the dams were present in Zenzele in May 2006 and may have been vaccinated by the DoA, however none had a titre suggestive of an anamnestic response to vaccination (defined as a peak titre ≥128 IU/ml (Morters and others 2014a)) in February 2010 (day 30 titres ranged from 0.09 – 90.5 IU/ml). The other two puppies (blood sampled on day 30) were obtained from outside Zenzele, therefore the vaccination status of their dams was not known. Only five of the (68) vaccinated puppies were still in Zenzele 90 days after vaccination, and of these four remained 12 months after vaccination (with day 360 titres of 0.09, 0.35, 0.35 and 1 IU/ml). This includes one puppy born in Zenzele that was not blood sampled 30 days following vaccination; however titres for

189 this puppy 90, 180 and 360 days following vaccination were ≥0.5 IU/ml. The dam of this puppy was
190 vaccinated with Rabisin in October 2009 with an anamnestic response to the vaccine (day 30 titre of
191 128 IU/ml), consistent with possible vaccination also in May 2006. See Table S1 for the pre- and
192 post- vaccinal titres of the (68) puppies and their dams.
193
194 All puppies in the Serengeti seroconverted to the vaccine at 21 days following vaccination, with all
195 titres exceeding 5.9 IU/ml (Table 2). In this site, no data were available for end-point titres,
196 vaccination status of the dams or survival of the puppies beyond the 21-day follow-up period. In the
197 Serengeti, where CDV and CPV were administered simultaneously with Nobivac Rabies, all puppies
198 also seroconverted to CDV and CPV with high 21-day post-vaccinal antibody titres of ≥256 and ≥640
199 respectively. See Table S2 for the pre- and post-vaccinal CDV and CPV titres.
200
201 At the time of blood sampling and the health assessment, there were no reports or clinical signs of
202 type IV hypersensitivity reactions (i.e. granulomas or sterile abscesses at the injection site), the main
203 risk associated with the use of inactivated vaccine with adjuvant (Merial Animal Health Limited ;
204 MSD Animal Health ; Tizard 2013). Although 14 puppies in Zenzele died before the first household
205 visit in March 2010 (see Table S1), none were reported to have died the day of vaccination,
206 suggestive of a type I hypersensitivity (anaphylactoid) reaction which may occur up to 2 or 3 hours
207 following vaccination (Tizard 2013); nor were there any reports of type IV hypersensitivity reactions.

Table 2 Day 0 (pre-vaccination) and day 21 (peak) titres of the puppies vaccinated in the Serengeti

dog	gender	age at vaccination (months)	day 0 titres (IU/ml)	day 21 titres (IU/ml)	ivermectin administered
1	m	3	0.17	>5.9	no
2	m	3	0.07	>5.9	yes
3	f	2	0.29	>5.9	yes
4	f	3	0.07	>5.9	no
5	m	3	0.07	>5.9	yes
6	m	3	0.07	>5.9	yes
7	f	2	0.07	>5.9	yes
8	f	3	0.17	>5.9	yes

208 **Discussion**

209

210 Our study shows that puppies from low-income communities in rabies endemic regions respond well
211 to a standard dose of high quality, inactivated rabies vaccine without any apparent adverse reactions
212 (Merial Animal Health Limited ; MSD Animal Health ; Tizard 2013). All the puppies sampled following
213 vaccination in this study generated antibody titres >0.5 IU/ml after vaccination, and most individuals
214 recorded much higher titres. Although the sample was small, somewhat related to the poor general
215 background survival of puppies here (see Table S1), this result was consistent across the study group
216 irrespective of the levels of pre-vaccinal antibody, the administration of ivermectin at the time of
217 vaccination, or concurrent vaccination against CDV and CPV. Nonetheless, given the lack of
218 published data, larger field studies to investigate the effects of ivermectin on immunological
219 responses to inactivated rabies vaccine may be warranted given that ivermectin is often
220 administered as part of rabies vaccination programs.

221

222 None of the puppies had pre-vaccination antibody titres >0.5 IU/ml that might be indicative of
223 maternal antibody against rabies. However, because of the uncertain vaccination status of the dams,

it was not possible to determine whether the low pre-vaccinal antibody titre of 0.29 IU/ml detected in one puppy in the Serengeti was the result of maternal antibody. More detailed studies of the maternal antibody status and immunological responses of puppies in these low-income settings is also warranted, particularly as the development of large-scale rabies control and elimination programmes across Asia and Africa (Lapiz and others 2012; Putra and others 2013; WHO 2013) is likely to result in an increasing proportion of puppies born to vaccinated dams.

Rabies is a serious zoonosis that remains uncontrolled in dog populations throughout much of Asia and Africa. Given the inadequacy of vaccination campaigns, a substantial proportion of free-roaming dogs in affected communities are never vaccinated or vaccinated only once in their lifetime (Lembo and others 2010; Mitmoonpitak and others 1998). Although mortality in puppies less than 3 months of age is generally high in these populations (Brooks 1990; de Balogh and others 1993; Gsell and others 2012; Kitala and others 2001; Morters and others 2014b), delaying vaccination until puppies are 3 months of age may result in these dogs never being vaccinated. On the basis of our results, and the risk of humans contracting rabies from young puppies (Awoyomi and others 2007; Mitmoonpitak and others 1997; Taiwo and others 1998; WHO 1998; Widdowson and others 2002), all dogs in rabies endemic regions, including puppies less than 3 months of age, should be vaccinated against rabies as recommended by the WHO (WHO 2004, 2013). While puppy vaccination should therefore be included in annual rabies vaccination campaigns, these efforts should not compromise vaccination of juvenile and adult dogs, which have higher survival rates than puppies and are therefore important in maintaining vaccination coverage between campaigns (Morters and others 2014b). As humoral immunity can wane rapidly in young dogs (Siegrist 2012) and puppies are continually acquired by community members throughout the year, it is recommended that all young dogs should also be given primary and booster vaccinations whenever veterinary services are available to dog owners.

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