ATTACHMENT

Available online at www.sciencedirect.com

ScienceDirect

Vaccine 25 (2007) 8500-8507

ELSEVIER

Vaccine

www.elsevier.com/locate/vaccine

Factors influencing the antibody response of dogs vaccinated against rabies

Lorna J. Kennedy [a'*] Mark Lunt [b], Annette Bames [c]
L01Taine McElhinney [d], Anthony R. Fooks [d],
David N. Baxter [e], William E.R. Ollier [a]

Centrefor Integrated Genomic Medical Research, University ofManchester, Stopford Building, Oxford Road, Manchester M13 9PT, UK

[b] ARC Epidemiology Unit, University ofManchester, UK

[c] Faculty Of Veterinary Science, University ofLiverpool, UK Veterinary Laboratories Agency, Weybridge, Surrey, UK

Greater Manchester Health Protection Unit, Peel House, Eccles, Greater Manchester, UK

see PAGE 110

Received 13 June 2007; received in revised form 11 September 2007; accepted 7 October 2007

Available online 26 October 2007

Abstract

Since 2000, there has been a legal requirement in the UK that dogs and cats should have an effective rabies vaccination with demonstrable sero-conversion if their owners wish to avoid quarantine on re-entry to the UK. In 2002, 10,483 rabies titres were determined on dogs at the VLA. Statistical analyses assessed the efficacy of each vaccine within different dog breeds. Animal size, age, breed, sampling time and vaccine had significant effects on pass rates and median titres. Our data suggests that a general relationshi between animal size and level of antibody re onse ler sized do elicited hi her antibo an ar f o It was not however, only the magnitude of response immediately following vaccination but also the duration of immunity that varied between breeds of dog. Another observation was that young animals, less than I-year of age, generated a lower antibody response to rabies vaccination than adults. Considerably higher failure rates were also observed for different vaccines tested. Regression analysis revealed that two vaccines performed equally well, and significantly better than the others tested. The variation in antibody response relating to length of interval of sampling following vaccination is not unexpected and presumably relates to the response kinetics for primary vaccination. These data need to be placed in perspective in order to minimise the risk of rabies being re-introduced into a rabies-free country, especially in the consideration of removing the requirement for serological testing for rabies vaccinated dogs that participate in pet travel schemes.

Keywords: Rabies; FAVN; Vaccine; Dogs; Pet travel scheme

1. Introduction

Rabies is a viral zoonosis transmitted to man, mainly by rabid dogs, although infection is also possible from bats and other mammals. There are an estimated 55,000 human deaths annually worldwide from rabies (http://www.who.int/ rabies/en/) and 94% of these are transmitted by rabid dogs [1]. Rabies virus belongs to the order Mononegavirales, which have non-segmented, negative stranded RNA genomes. The virus infects the central nervous system causing encephalopathy followed by rapid death. Prevention of rabies in humans can be achieved by vaccination either by pre or post exposure prophylaxis.

In the UK, the pet travel scheme (PETS) was introduced to enable pets (primarily dogs and cats) to be brought into the UK without a lengthy quarantine. (http://www.defra.gov-uk/ animalh]quarantine/pets/index.htm). It also allows pets to be taken outside the UK and re-enter without quarantine (e.g. to be taken on holiday with its owners). An important part of this scheme is the requirement to ensure protection from rabies by vaccination, which in the UK is achieved using inactivated vaccines. Until the introduction of PETS, the rabies-free status Of the UK was effectively controlled by long quarantine periods [2]. There are a number of companies who manufacture rabies vaccines and the standard method of testing to check whether a dog has adequate immunological protection is by measuring virus neutralising antibodies (VNA) using the fluorescent antibody virus neutralisation test (FAVN) [3], which measures both IgM and IgG, performed at officially recognised laboratories. A serum titre of 0.5 Wml and above ofrabies virus-specific antibodies is considered adequate protection against rabies. A titre below this level is considered a vaccination "failure", leaving the dog less likely to be protected from the rabies virus [2,4]. It is becoming increasingly apparent that not all dogs respond equally to vaccination. A proportion fails to develop a protective immune response, leaving them susceptible to disease [5]. By contrast, some animals appear to "over" respond to vaccines potentially leading to adverse reactions [6,7], such as Arthus type 3 hypersensitivity reactions when re-vaccinated. Variations in vaccine response may in part be a genetically pre-programmed event and this is compatible with anecdotal reports of immune response differences between breeds. A previous analysis of samples submitted for antibody testing, demonstrated that vaccine type, sampling interval, age and origin of animal all significantly affected the failure rate [8].

The immune response is probably the best example of a complex continuous trait where multiple genetic, environmental and lifestyle factors all contribute and interact to produce the phenotype measured. One strong genetic factor known to influence immune response to vaccination is the Major Histocompatibility Complex (MHC). We have previously identified that dog leukocyte antigen (DLA) polymorphism is related to both autoimmune susceptibility and infectious disease susceptibility [9—13]. Our intention is to examine DLA gene polymorphism in rabies vaccination. However, before attempting to investigate the relationship between canine immune response to vaccination and gene polymorphisms, it is necessary to identify the factors which correlate with antibody production in a primary response. A dataset relating to a cohort of dogs vaccinated against rabies has provided this opportunity.

* Corresponding author. Tel.: +44 161 275 7316; fax: +44 161 275 1617. E-mail address: Loma-Kennedy@manchester.ac.uk (LJ. Kennedy).

0264-410X/S — see front matter 0 2007 Elsevier Ltd. All rights reserved.
d0i:IO.1016fj.vaCCine.Z007.IO.015

2. Material and methods

Serological and basic demographic data from a cohort of 10,483 dogs tested at the Veterinary Laboratory Agency (VLA) for rabies antibody during 2002 were available for analysis. The dataset included dog breed, sex, age, vaccine manufacturer, vaccine batch used, date of vaccination, date of sampling for antibody titre and antibody titre. All testing for rabies antibodies was undertaken at the Veterinary Laboratories Agency (VLA), Weybridge UK using the FAVN test- A titre of less than 0.5 IU/mI was considered a vaccination failure. The dataset was assessed for errors, such as mistyped dates, and these records were excluded from further analysis. The six vaccine manufacturers were Fort Dodge, Intervet, Merial, Schering-Plough, Virbac and other (mainly unknown). For the analysis these were coded A—F, but not in this order.

2.1. Statistical methods

We considered the effect, on both the success of the vaccination and the titre, of the following variables: vaccine source, vaccine batch, age, sampling time (i.e. the number of days between the date of vaccination and the date of a sample being taken for antibody titre testing), size and breed. Logistic regression was used when considering

success, linear regression when considering the titre (after log-transforming the data to obtain a normal distribution). Size, breed, vaccine source and vaccine batch were fitted as categorical variables. To allow for the possibility of non-linear associations between continuous predictors and outcomes, polynomial terms were included in the models, with additional terms being added until the last term added was not statistically significant. The predicted values from these regression equations were compared to the mean values at each age (for ages $15, for which there were sufficient numbers of dogs to do so) to assess the goodness of fit of the models.

To investigate the effect of vaccine batch on failure rate, a logistic regression model was fitted with an indicator variable for each batch. The most similar batch coefficients were constrained to be equal, and the process continued until any further simplification of the model led to a statistically significant reduction in the fit of the model.

All analyses were performed using Stata (StataCorp, 2003). To get an idea of the distribution of the titres, a kernel density estimator, a form of smoothed histogram, was used [14]. Box and whisker plots were produced for each breed and vaccine separately, if there were at least 16 dogs of that breed vaccinated with each vaccine. These plots consist of boxes showing the median, the 25th percentile and the 75th percentile. The whiskers extend to the most distant observation which is less than 1.5 x the inter-quartile range. Any individual observation outside this range is plotted separately.

3. Results

Data were assessed both in terms of success/fail rates, plus absolute and median titres for all the analyses conducted. The majority of animals had been vaccinated with vaccines A (n = 5272) and B (n = 4313). The remaining animals were vaccinated with vaccines C (n = 481), D (n = 184), E (n = 115) and F 116).

The overall failure rate for the different vaccines varied from 0.01 to 0.20, (Fig. I). While all four batches of vaccine C had higher failure rates than vaccines A or B, one particular batch had a failure rate of 32.9% (data not shown), which was only used for 70 dogs. Only data for vaccines from A and B were included in further analysis due to the vastly smaller

1 06

8502

LJ.

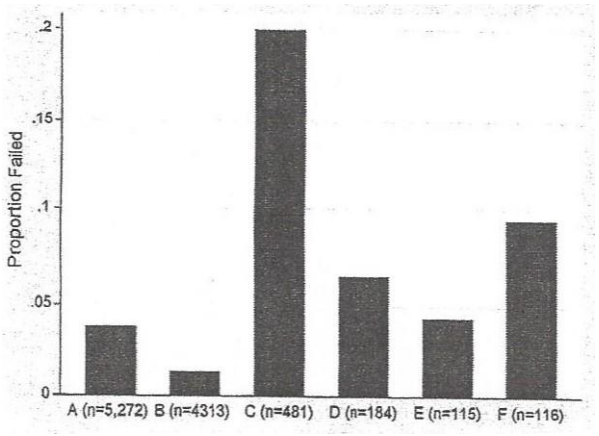


Fig. 1. Percentage of failures by vaccine,

numbers in the other groups. On average vaccine B resulted in a five times higher antibody titre than vaccine A. This can be seen in Fig. 2, which represents kernel density estimates for the titres produced by the two vaccines.

Batch variation within vaccines A and B was also considered. Logistic regression indicated that the 18 different vaccine A batches could be clustered into 2 different groups, with a mean failure rate of 3% in one group and 9% in the other. Vaccine B showed no significant between-batch (n = 22) variation (data not presented).

Fig. 3a shows the proportion that failed by age. This graph fits the predicted values from a logistic regression equation and the observed proportion failing at each age. Since small differences in vaccination failure rates might have a disproportionately large effect where the total number in that age group is small, dogs aged over 15 years of age (n = 49) were excluded from the analysis.

When vaccinated dogs were divided into three age groups: young (<1 year), adult (1—7 years) and old (>7years), logistic regression showed that both young and old dogs were significantly more likely to fail than adult dogs. Old dogs were

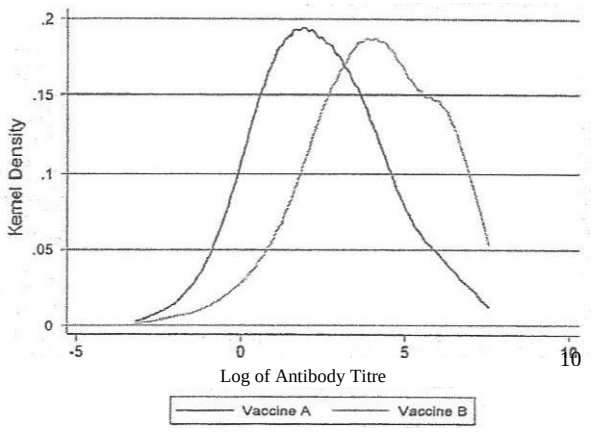


Fig- 2. Log of antibody response for vaccines A and B.

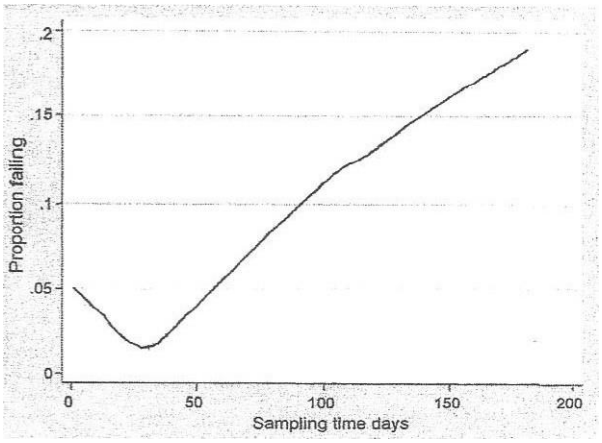


Fig. 4. Percentage of failures by sampling time.

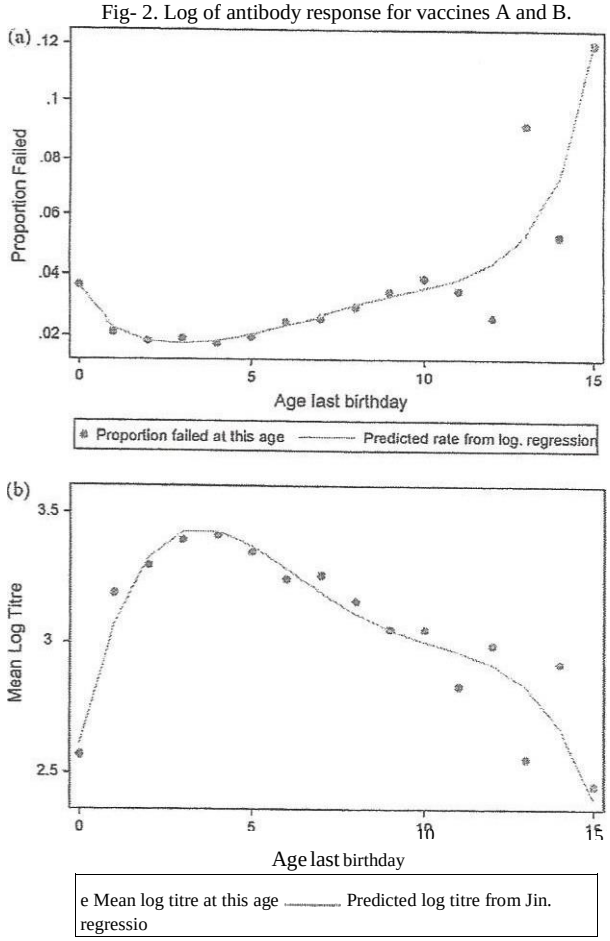
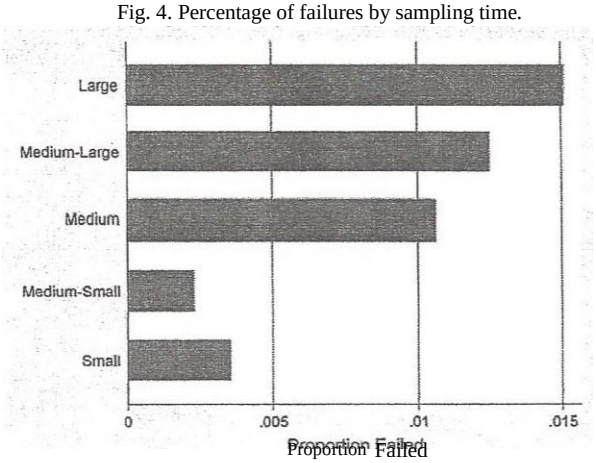


Fig. 3. (a) Percentage failure by age and (b) average titre by age.



more likely to fail using both vaccines ($p < 0.04$ for vaccine A, $p < 0.001$ for vaccine B), but young dogs were only more likely to fail when using vaccine A ($p<0.001$ vs. $p <0.3$). Adult dogs also had statistically significantly higher titres than young and old dogs, (see Fig. 3b). The highest titres occurred in dogs approximately 3-4 years of age. Gender

Fig. 5. Proportion of dogs failing rabies vaccination according to size cate-

had no effect on failure rate or titre, but, interestingly, neutering increased the titre for both vaccines by 22% (data not shown). A plot of the proportion that failed against sampling time shows that the least failures occur at 30 days (Fig. 4). When animals were divided into three sampling time groups: short (<20 days) nonnal (20—50 days) and long (>50 days), analysis showed that the usual 28 day sampling time gave significantly lower failure rates (2<0.00001) than short or long sampling times.

Weight was not recorded in the dataset, and consequently an estimated weight and height category (small, medium and large) was assigned to each dog based on breed standards. These were combined into five size categories: small, small—medium, medium, medium—large and large. Data were then analysed for variation attributable to size category. Failure rate increased with increasing size ($p < 0.007$), apart from the small and medium—small groups, which were in reverse order, (Fig. 5), as did median antibody titres decreased (data not shown). These differences were significant except for the lowest two groups.

Fig. 6 shows the distributions of titres using vaccines from both vaccines A and B for all breeds that had at least 16 dogs vaccinated with either of the two vaccines. The black vertical line shows the threshold for the vaccination being considered a success. As previously mentioned, titres were higher for vaccine B than vaccine A and this was seen in all breeds. However, the titre observed is smaller in the larger dogs. Although most of the failures are in the larger breeds (e.g. German shepherd dogs and Labradors), there are also some smaller breeds with important failure rates (Cavalier King Charles spaniels and west highland white terriers).

We also calculated an average titre for each breed, and then divided the titres into quartiles. If size accounted for all the variation, then the Lowest quartile should include the large breeds and the highest quartile should include only small breeds. Breeds were listed by quartile and size,

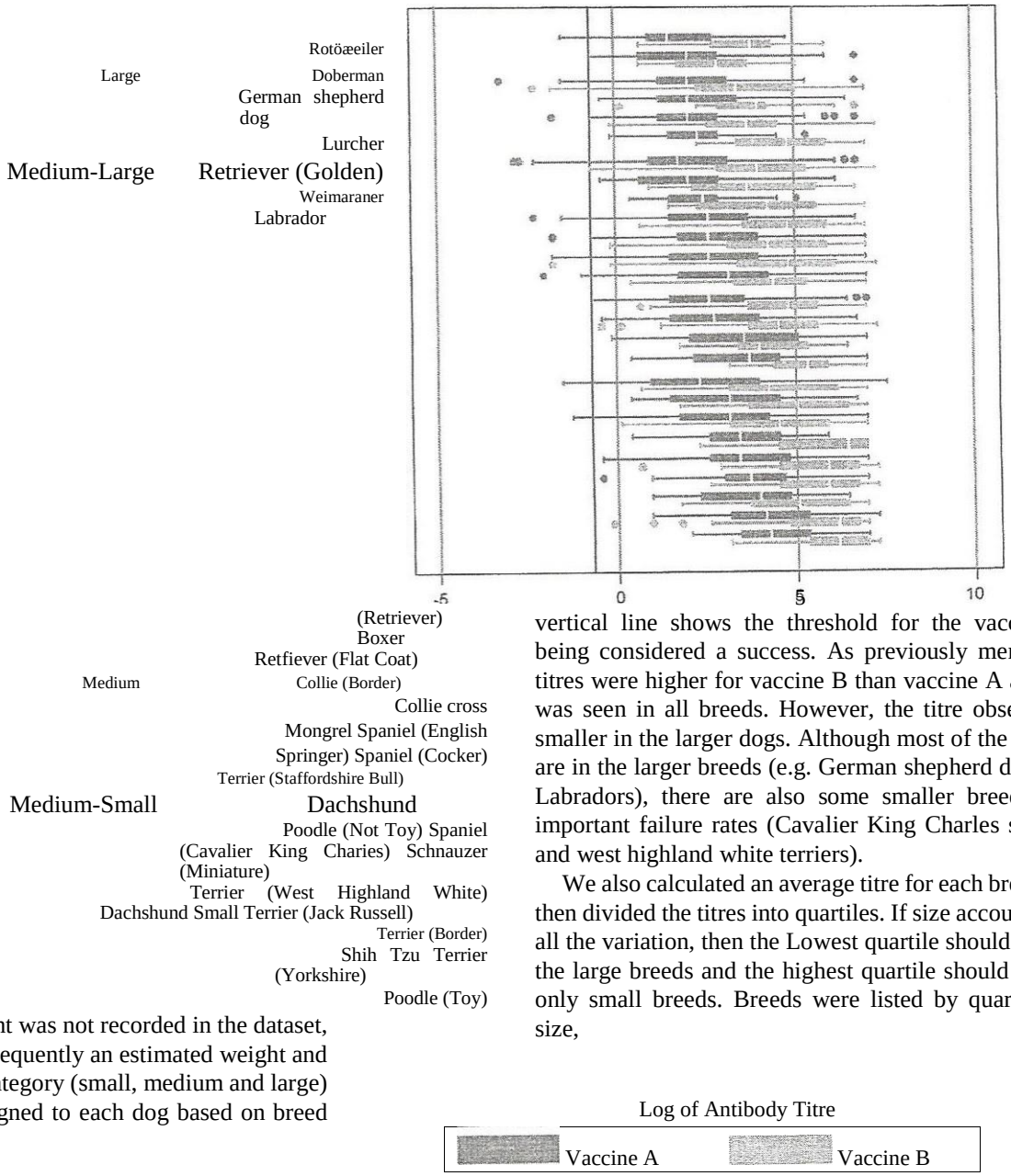


Fig. 6. Box and whisker plots of titres of vaccines A and B for all breeds with at least 16 dogs vaccinated with each of the 2 vaccines-

Table 1
Breeds listed as uartile by titre against size

Breed	anile	titre		Medium—I	Medium	Medium—small	Small
			17				
Bernese Mountain dog	19						
Akita (Japanesc)							
Great Dane							
Rhodesian Ridgeback							
			71				
Rottweiler	101						
Setter (Red)							
Retriever (Golden)				311			
German shepherd dog							
Collie (Rough)							
Collie (Bearded)					49		
Labrador (Retriever)							
Bulldog							
Basset hound							
Sheepdog (Shetland)							
Terrier (Scottish)							
Malamute (Alaskan)			13				
Bouvier des Flandres			17				
Newfoundland			28				
Setter (Gordon)				19			
Setter (English)	31 Greyhound	47					
Shar Pei							
Husky (Siberian)					18		
Spaniel (Welsh Splinger)							
Pointer (German)							
Vizsla (Hungarian)							
Sheepdog (Old English)	37 Terrier (Airedale)	39					
Dalmatian							
Retriever (Golden)							
Boxer					157		
Collie cross	249 Collie (Border)	434					
Beagle							
Spaniel (Cocker)							
Terrier (Norfolk)							18
Terrier (Patterdale)							
Spaniel (Cavalier King Charis)							
Weimaraner							
Mongrel	1,417 Spaniel (English Springer)	320					
Terrier (Bull)							
Terrier (West Highland White)							338
Pointer							
W*hippet							
Terrier (Scottish)							
Terrier (Tibetan)							
Dachshunda						61	58
Poodlea						128	59
Terrier (Staffordshire Bull)						178	
Spaniel (King Charles)	19 Terrier (Lakeland)	19					
Pug							
Tenier (Maltese)							
Pekingese							
							27
Papillon							
Chihuahua							
Terrier(Cairn)							
Lhasa Apso							81
Schnauzer (Miniature)							
							108
Shih Tzu							
							110
Bichon Frise							
							124
Terrier(Border)							
							284
Terrier (Yorkshire)							
							506
Terrier (Jack Russell)							

Breeds in bold text are not in the expected ordez

a Poodles and Dachshunds are shown as a •ingle group but there are medium—large and miniature (toy) varieties, which were not differentiated in our analysis.

and it is clear that some breeds were not in their expected position (see breeds in bold text in Table I). For example, two small breeds (Shetland sheepdog and Scottish terrier) plus two medium—small breeds (Basset hound and Bulldog) were classed in the lowest quartile of titre. Similarly three small breeds (Cavalier King Charles spaniel, Patterdale terrier and Norfolk terrier) plus two medium small breeds (Cocker spaniel and Beagle) were in the second quartile. These can be considered to be poor responders. It is interesting to note that beagles are usually used for vaccine trials and registration of vaccine products and yet there are clear differences between responses in this breed and other pedigree dogs. The choice of dog breed in vaccine immunity studies should therefore be a careful consideration. At the other end of the spectrum the large breed Weimaraner was in the third quartile, while Pointers (medium—large) and Whippets (medium) were in the highest quartile, which can be considered as good responders. The breed identity supplied on sample submission forms may not always be accurate and a small proportion of the dogs stated to be a certain breed will not be pedigree animals and therefore would not necessarily conform to the "breed standard". We recognize that this may introduce some errors into the dataset, however, we do not consider this to be significant to alter the overall conclusions of the data.

Of the variation in log titre observed, 19% was due to differences between vaccine A and vaccine B, and a further 8% due to differences between breeds. Ofthe differences between breeds, 5% could be attributed to differences in size between the breeds, and 3% to other differences. These other differences remain highly statistically significant ($p < 0.0001$).

4. Discussion

We have analysed a large dataset of dogs vaccinated against rabies as part of the pet passport scheme. This analysis raises a number of important issues relating to canine vaccination in general and rabies vaccination in particular. Vaccines produced by different manufacturers have significantly different failure rates, and significantly different median titres of response. This is presumably due to their formulation and the production differences between vaccines together with the concentration and integrity of antigen content and the adjuvant used. Although such differences occur it is difficult to fully assess how clinically meaningful this is, if all commercially available vaccines generate suffcient immunity in most animals.

Interestingly, analysis of the most frequently used vaccines (A and B) revealed a bimodal distribution of response with a small group of dogs making higher antibody levels. An explanation for this observation can only be speculated at. It is possible that within the animals vaccinated there is a distinct group of dogs which make a higher antibody response due to their genetic profile or other characteristics such as size or age, since size, age of the dog and the time interval between vaccination and sampling for rabies antibody titre all had significant effects on failure rates and median titres-

ody response clearly exists [8]. This could be ex lained

A general relationship between animal size and le an vaccme ose-e ect, as It is unclear whether an standard or adjusting vaccine dose werg t and whether small an argedogsreceive the same vo ume o Injection. However this seems an unlikely as the immune system of an ani-

mal encounters suffcient antigen to make a response, larger doses of antigen are not a major factor in increasing antibody production in primary responses. An alternative explanation could be that larger dogs are more likely to have deeper subcutaneous fat at popular sites for injection. Deposition and sequestration of antigen in fat is known to reduce the level of immune response [15,16] Any studies to clarify such potential variability and standardize procedures would clearly be beneficial in improving animal welfare.

The relationship between antibody response and age is also an important consideration. The observation that young animals (<1 year) make a poorer immune response to rabies vaccination than adults could be due to their immune systems being less mature. A reduction in immune response in the elderly is well documented in humans [17] even though overall immunoglobulin levels tend to rise with age, as does the prevalence of auto-antibodies [18]. A reduction in immune regulation is thought to occur with age and this may explain why older dogs have a poorer response to rabies vaccination. A policy of further booster vaccination may be justified for older dogs although this would need careful study i.e. a trial. Another complicating factor is the different life expectancies for different breeds, which makes the definition of "old" vary for different breeds.

The variation in antibody response relating to length of interval of sampling following vaccination is not unexpected and presumably relates to the response kinetics for primary vaccination. Dogs iso-type shift from an IgM response to an IgG as an immune response develops, and optimum measurement in an appropriate window of time should measure this effect. Measurement at too early a stage will predominantly only capture an IgM response developing but not be able to confirm whether an IgG response progresses. Measurement only at a later time point may reveal lower antibody levels, but this may not relate to a lack

of immune protection as the total immunoglobulin measure may be proportionately more accounted for by IgG.

This study clearly demonstrates that the standard time frame for sampling following vaccination should be adhered to by oWners and veterinarians if a true picture of rabies immunity is to be determined. In Europe with quarantine and PETS it would appear that rabies is under control. However on a global level, rabies is still an ir-nportant health issue. This study raises many variables that need further investigation and explanation. For example the time frame for vaccination and serological testing, the variable failure rates of some vaccines, the wide range of antibody responses and, interestingly, the variability within and between breeds in response to vaccination. We should therefore not become complacent about the spread of rabies within Europe and further work is clearly required. In some parts of the world rabies is not under control and a better understanding of the immune response to vaccination would help in devising strategies especially in the feral populations. A clear correlation exists between the reduction of human cases and the reduction of cases in the domestic dog, consequently disease control in animals must

L

be effective [19]. The possibility that in 2008, the UK, under an EU directive, will not have serological testing could therefore be premature given our current level of understanding. A risk assessment carried out by Defra also supports this stance [20].

Current differences exist between the UK and Sweden with their respective travel schemes in that serological testing for entry into the UK was set at an optimum of 28 days although it was still possible to take an earlier blood sample if deemed necessary with the understanding that the titre might be low. In contrast, in Sweden the date of blood sampling was set at 120 days following vaccination to ensure that dogs with high titres still had a measurable titre after this time period. Hence, Sweden had a much higher failure rate than the UK. As the blood sample was only taken from a single time point, it was argued by the cornpetent authorities in the UK that the titre level was only a marker of seroconversion to the vaccine and not an indication of protection. In addition, the vaccines used had proven efficacy and vaccine potency. This assumed that the minimum antibody threshold (0.05 IU/mI) was attained. If, during the 3-year period before a booster vaccination was required, it was assumed that memory B- and T-cells had been primed and an anamnestic response would generate VNA in response to a viral challenge.

In the US vaccinated dogs and cats regularly succumb to natural rabies, probably from a wildlife reservoir [21]. This would suggest that there is an increased risk, if vaccinated non-responders are allowed into rabies-endemic areas.

Interestingly, considerably higher failure rates were observed for vaccine A (17.1%) than vaccine B (3.5%) for samples taken >50 days post vaccination. One interpretation of this and the data suggested in Fig. 3a is that vaccine B provokes better sero-conversion to IgG responses. Although vaccine A has relatively low fail rates at sampling ümes <20 days and 20—50 days, this becomes higher at times >50 days as presumably IgM levels diminish.

After these factors were excluded, residual failure rates of 3.0% and 0.7% remain (vaccines A and B, respectively). These data represents a high level of risk in the consideration of removing the requirement for testing that rabies vaccination has been successful.

Analysis restricted to only adult dogs with a normal sampling time revealed that size still has a significant effect on vaccine response, and accounts for approximately half the differences between breeds. However, significant differences between breeds still exist. Importantly, even by taking breed and size into account, other factors must explain some of the differences in response between dogs, including failures.

We know from our extensive DLA typing of over 4000 dogs, that some breeds have characteristic distributions of DLA polymorphisms and may also have very restricted DLA allele and haplotype profiles [22—24]. Two breeds that respond poorly to both vaccines are Doberman and Rottweiller, both with low DLA variation, having just four and two major haplotypes respectively. There is some sharing of DLA alleles between Doberman and Rottweiller. In contrast, Shih Tzu, which respond well to both vaccines also have a fairly restricted profile of five DLA haplotypes, two of which have only been found in Shih Tzu to date, and with little overlap for the alleles found in Dobermanns.

From our preliminary analyses a wide range of antibody response exists for most breeds, even when only adult dogs tested in the optimum time frame are considered. This could be explained by allelic variation of DLA class Il loci in breeds. It is possible that low and high levels of response are associated with particular DLA polymorphisms. Although MHC genes are known to play a major role in determining the level of immune response, the response to an antigen is undoubtedly under complex genetic control. Other gene polymorphisms such as those in IL-IO and other THI/TH2 regulatory cytokines may also contribute to this regulation. This control of vaccine response will apply to all vaccines, not just rabies.

We now intend to investigate the role of DLA and other immuno-regulatory cytokine gene polymorphisms in determining canine response to vaccination.

Acknowledgements

ARF and LMcE were funded by the UK Department for Environment, Food and Rural Affairs (Defra) by grant SEV3800. We are grateful to Mrs. Trudy Goddard for carrying out the FAVN tests.

References

[1] Perrin P, Jacob Y, AguiIar-Setien A, Loza-Rubio E, Jallet C, Desmezieres E, et al. Immunization ofdogs with a DNA vaccine induces protection against rabies virus. Vaccine 1999; 18(5—6):479—86.

(2] FooksAR, McElhinneyLM, Brookes SM,JohnsonN, Keene V, Parsons G, et al. Rabies antibody testing and the UK pet travel scheme. Vet Rec 2002;150(14):428–30.

[3] Cliquet F, Aubert M, Sagne L Development of a fluorescent antibody virus neutralisation test (FAVN test) for the quantitation of rabiesneutralising antibody. J Immunol Meth 1998;212(1):79—87.

[4] Burr PD, Snodgrass DR. Serological response to rabies vaccination for the pet travel scheme. Res Vet Sci2002;72(S

[5] Ek-Kommonen C, Sihvonen L, Pekkanen K, Rikula U, Nuotio L Outbreak of canine distemper in vaccinated dogs in Finland. Vet Rec 1997;141(15):380–3.

[6] Tizard IR, Vaccination, Vaccines.Veterinary immunology. 5th ed. London: WB- Saunders; 1996.

[7] Tjalve H. Adverse reactions to veterinary drugs reported in Sweden during 1991-1995. J vet Pharmacol Ther1997;20(2)

[8] Mansfield KL, Burr PI), Snodgrass DR, Sayers R, Fooks AR. Factors affecting the serological response of dogs and cats to rabies vaccination. vet Record2004;1

[9] Kennedy LJ, Davison LJ, Barnes A, Isherwood D, Oilier WER, Catchpole B. Susceptibility to canine diabetes mellitus is associated with MHC class Il polymorphism. BSAVA annual congress 2003 scientific proceedings 2003. p. 593.

[10] Kennedy LJ, Huson HJ, Leonard J, Angles JM, Fox LE, Wojciechowski JW, et al. Association of Hypothyroid disease in Doberman Pinscher dogs with a rare Major Histocompatibility Complex DLA class haplotype. Tissue Antigens2006;67:

[I I] Kennedy LJ, Quarmby S, Happ GM, Barnes A, Ramsey 1K, Dixon RM, et al. Association of canine Hypothyroidism with a common Major Histocompatibility Complex DLA class Il allele. Tissue Antigens 2006;68:82—6.

[12] Oilier WER, Kennedy U, Thomson W, Barnes AN, Bell SC, Bennett D, et al. Dog N'IHC alleles containing the "human RA shared epitope" confer susceptibility to canine rheumatoid arthritis. Immunogenetics 2001;53:669–73.

[13] Quinnell RJ, Kennedy LJ, Barnes A, Courtenay O, Dye C, Garcez LM, et al. Susceptibility to visceral leishmaniasis in the domestic dog is associated with MHC class 11 polymorphism. Immunogenetics 2003;55(1):23–8.

[14] Fox J. Describing univariate distributions. In: Fox J, Long JS, editors. Modern methods of data analysis. Newbury Park, CA: Sage Publicadons; 1990. p. 58-125.

[15] Ellis RW, editor. Hepatitis B vaccines in clinical practice. Marcel Dekker; 1993.

[161 Keating GM, Noble S. Recombinant hepatitis B vaccine (Engerix-B): a review of its immunogenicity and protective effcacy against hepatitis B. Drugs2003;63(10):1021–51.

[17] Aspinall R. Longevity and the immune response. Biogerontology 2000;1(3):273–8.

[18] Tomer Y, Shoenfeld Y. Ageing and autoantibodies. Autoimmunity 1988;1(2):141–9.

[19] Rupprecht CE, Hanlon CA, Hemachudha T. Rabies re-examined. Lancet Infect Dis2002;2(6):327–43.

[20] Wilsmore T, Hamblin C, Taylor N, Taylor W, Watson B. Qualitative veterinary risk assessment of the introduction or rabies into the United Kingdom. A report prepared for Defra (Department for the Environment, Food and Rural Affairs) 2006: http://www.defra.gov.uW animalh/diseases/notifiable/rabies/pdffqra-rabies .pdf.

[21] Eng TR, Fishbein DB. Epidemiologic factors, clinical findings, and vaccination status of rabies in cats and dogs in the United States in 1988. National Study Group on Rabies. J Am Vet Med Assoc 1990;197(2):201–9.

[22] Kennedy L], Barnes A, Happ GM, Quinnell RJ, Bennett D, Angles JM, et al. Extensive interbreed, but minimal intrabreed, variation of DLA class Il alleles and haplotypes in dogs. Tissue Antigens 2002;59(3):194–204.

[23] Kennedy LJ, Barnes A, Happ GM, Quinnell RJ, Courtenay O, Carter SD, et al. Evidence for extensive DLA polymorphism in different dog populations. Tissue Antigens 2002;60(52.

[24] Kennedy U, Carter SD, Bames A, Bell S, Bennett D, Oilier WER, et al. Interbreed variation of DRB I , DQAI alleles and haplotypes in the dog. Vet Immunol Immunopathol1999;69: