Rabies Antibody Titres in Vaccinated Dogs

J.B. DERBYSHIRE AND K.A. MATHEWS

Department of Veterinary Microbiology and Immunology (Derbyshire) and Department of Clinical Studies (Mathews), Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1

SUMMARY

In a field study, rabies virus neutralizing antibody titres were determined by the microtest modification of the rapid fluorescent focus inhibition test before and after primary vaccination in 30 puppies, and before and after booster vaccination in 59 previously vaccinated dogs. A commercial modified live virus vaccine was used. Three weeks after primary vaccination the mean antibody titre was 102 ± 90, but only 24 dogs presented for booster vaccination had detectable antibody levels (mean titre 12 ± 16). The antibody responses three weeks after booster vaccination (mean 380 ± 216) were significantly greater than the responses to primary vaccination. It was concluded that previously vaccinated dogs could have an anamnestic response to booster vaccination, even when antibodies were not detected in their sera before revaccination.

Key words: Rabies, rabies vaccine, vaccination, dogs.

RÉSUMÉ

Titres d'anticorps antirabiques, chez des chiens vaccinés

Cette étude clinique visait à déterminer le titre d'anticorps antirabiques neutralisants, à l'aide d'une microtechnique correspondant à une modification de l'épreuve rapide de l'inhibition des foyers de fluorescence, avant et après la vaccination initiale de 30 chiots, ainsi qu'avant et après la revaccination de 59 chiens. Les auteurs utilisèrent à cette fin un vaccin commercial atténué. Trois semains après la vaccination initiale, le titre moyen d'anticorps atteignit 102 ± 90, mais seulement 24 des chiens soumis à la revaccination possédaient un titre décelable d'anticorps dont la moyenne se situait à 12 ± 16. Trois semaines après la revaccination, le titre moyen d'anticorps atteignait 380 ± 216 et se révélait donc significativement plus élevé qu'après la vaccination initiale. Les auteurs conclurent que les chiens déjà vaccinés pourraient présenter une réponse immunitaire secondaire, même s'ils n'affichent pas d'anticorps décelables, avant leur revaccination.

Mots clés: rage, vaccin antirabique, vaccination, chiens.

INTRODUCTION

The relatively high prevalence of sylvatic rabies in Southern Ontario has led to increased exposure of dogs to infection, with consequential high quarantine costs. The need to quarantine vaccinated dogs has been questioned by veterinary practitioners and dog owners. While a range of rabies vaccines is available in Canada for use in dogs (1), few data are available on the immune status of dogs vaccinated under field conditions, although there are several reports in the literature on duration of immunity studies in dogs under laboratory conditions (2,3,4,5,6). The objectives of the study reported in this paper were to determine the rabies antibody titres of previously vaccinated dogs when presented for booster vaccination, and to compare the serological response to booster vaccination with the response to primary vaccination. The vaccine selected was a widely used modified live virus product, the immunogenicity of which has been well documented in laboratory studies (2,4).

MATERIALS AND METHODS

Vaccination and Collection of Sera

Fifty-nine previously vaccinated dogs were presented for a booster dose of vaccine at the Ontario Veterinary College Teaching Hospital during the period of the study. Each dog was blood sampled immediately before vaccination, and a second sample was collected from each dog three weeks later. Most of the dogs had been vaccinated between one year and two years previously, since annual revaccination is recommended in this high risk rabies area. Thirty puppies between three and four months of age received primary vaccination during the same period. Each was blood sampled before and after vaccination as above. The rabies vaccine used (4) was a commercial modified live virus vaccine prepared from the high egg passage Flury strain of rabies virus cultivated in an established canine kidney cell line (Rabies Vaccine Endurall-R, Norden, Lincoln, Nebraska 68501). The vaccine was administered according to the manufacturer's directions.

Rapid Fluorescent Focus Inhibition Test

The sera were stored at -20°C, and heat inactivated at 56°C for 30 min before being tested. Each serum sample was tested for rabies virus neutralizing antibodies by the microtest modification of the rapid fluorescent focus inhibition test (RFFIT), essentially as described (7), with the modifications outlined below. The virus used in the test was a derivative (ERA-Hpp) of the ERA strain (8) of rabies virus which had been plaque purified by Drs. R.B. Stewart and M.V. O'Shaugnessy of Queen's University, Kingston, Ontario and which was kindly supplied for our use by Mr. F.J. DePauli, Department of Veterinary Microbiology and Immunology, University of Guelph.

Supported by a grant from the Canadian Veterinary Research Trust Fund.

The virus was cultivated in BHK-21 cells, concentrated and partially purified by density gradient ultracentrifugation and used in the test at a working dilution of 1:50. The plates were examined with a Leitz Wetzlar Ortholux microscope with epifluorescence. The positive control serum used in the test was kindly supplied by Dr. J.B. Campbell, Department of Microbiology, University of Toronto and the serum titres were calculated as described (7). Mean antibody titres were compared by the t-test at the 5% level of significance.

RESULTS

Antibody Titres in Dogs Presented for Primary Vaccination

The titres are shown in Table I. Rabies antibodies were not detected in any of the dogs before vaccination. The postvaccination titres ranged from 16 to 305, with a mean and SD of 102 ± 90. The postvaccination titres were 32 or greater in 77% of the dogs.

Antibody Titres in Dogs Presented for Booster Vaccination

The titres are shown in Table II. Of the 59 dogs tested before vaccination, antibodies were not detected in 35. The prevaccination titres in the remaining 24 dogs ranged from 4 to 83, with a mean and SD of 12 ± 16. The postvaccination titres ranged from 27 to 724, with a mean and SD of 380 ± 216. A postvaccination titre of 32 or greater was found in 98% of the dogs. The mean response of these dogs was shown by the t-test to be significantly greater than the mean response of dogs to primary vaccination. However, the response of dogs which had residual titres before booster vaccination (mean postvaccination titre of 395 ± 227) was not significantly different than the response of dogs which lacked residual titres before booster vaccination (mean postvaccination titre of 323 ± 234). The mean response of the dogs which lacked residual antibody titres before booster vaccination was significantly greater than the response of dogs to primary vaccination.

TABLE I
RABIES ANTIBODY TITRES[a] IN DOGS PRESENTED FOR PRIMARY VACCINATION

Dog No.	Pre-vaccination Titre	Post-vaccination Titre	Dog No.	Pre-vaccination Titre	Post-vaccination Titre
P1	< 4	128	P16	< 4	16
P2	< 4	128	P17	< 4	64
P3	< 4	128	P18	< 4	128
P4	< 4	16	P19	< 4	117
P5	< 4	64	P20	< 4	16
P6	< 4	99	P21	< 4	305
P7	< 4	76	P22	< 4	64
P8	< 4	54	P23	< 4	305
P9	< 4	32	P24	< 4	305
P10	< 4	64	P25	< 4	128
P11	< 4	128	P26	< 4	305
P12	< 4	54	P27	< 4	16
P13	< 4	108	P28	< 4	16
P14	< 4	108	P29	< 4	16
P15	< 4	64	P30	< 4	16

[a]Antibody titres in the microtest modification of the RFFIT, calculated as described (7).

TABLE II
RABIES ANTIBODY TITRES[a] IN DOGS PRESENTED FOR BOOSTER VACCINATION

Dog No.	Pre-vaccination Titre	Post-vaccination Titre	Dog No.	Pre-vaccination Titre	Post-vaccination Titre
B1	< 4	724	B31	5	609
B2	83	305	B32	< 4	27
B3	< 4	256	B33	< 4	215
B4	19	181	B34	< 4	256
B5	< 4	609	B35	10	609
B6	< 4	664	B36	4	305
B7	< 4	64	B37	5	609
B8	< 4	181	B38	< 4	32
B9	< 4	305	B39	4	362
B10	< 4	609	B40	6	512
B11	6	724	B41	< 4	512
B12	< 4	181	B42	< 4	215
B13	< 4	181	B43	< 4	609
B14	6	181	B44	< 4	609
B15	8	305	B45	< 4	431
B16	23	724	B46	< 4	724
B17	23	108	B47	< 4	609
B18	< 4	128	B48	6	512
B19	4	128	B49	< 4	558
B20	6	512	B50	4	431
B21	< 4	99	B51	< 4	305
B22	< 4	362	B52	6	215
B23	< 4	256	B53	< 4	152
B24	< 4	431	B54	6	64
B25	6	512	B55	6	181
B26	6	664	B56	< 4	99
B27	< 4	609	B57	< 4	664
B28	< 4	609	B58	4	512
B29	< 4	32	B59	< 4	609
B30	23	215			

[a]Antibody titres in the microtest modification of the RFFIT, calculated as described (7).

DISCUSSION

The responses of the puppies in our study at three weeks postvaccination were regarded as satisfactory, and they were generally of a similar order to those recorded in dogs vaccinated under laboratory conditions (4), while the antibody titres of the dogs presented for booster vaccination were somewhat lower than might have been anticipated from the results of the earlier laboratory study (4). Some of the reasons for variability in the immune response of dogs vaccinated under field conditions have been discussed elsewhere (9), and a recent

study in Switzerland, where rabies vaccination is compulsory, revealed relatively low antibody titres in 20-25% of the dogs tested (10). However, the dogs in our study showed evidence of an anamnestic response to booster vaccination, even when antibodies could not be detected in their sera before revaccination. It has also been demonstrated (4) that vaccinated dogs which lack detectable rabies antibodies may resist challenge. While it is highly likely that almost all previously vaccinated dogs will be more resistant to field exposure than unvaccinated dogs, it would appear premature to propose relaxation of the quarantine procedures for such dogs unless arrangements could be made to determine their antibody status close to the time of exposure, and in response to any subsequent administration of vaccine which might be recommended.

ACKNOWLEDGMENTS

Financial support from the Canadian Veterinary Research Trust Fund is gratefully acknowledged. We wish to thank our colleagues in the Department of Clinical Studies for their cooperation in collecting serum samples from the dogs. Hazel Eaglesome and Nigel Gumley provided skilled technical assistance.

REFERENCES

1. VETERINARY BIOLOGICS STAFF, HEALTH OF ANIMALS DIRECTORATE, FOOD PRODUCTION AND INSPECTION BRANCH, AGRICULTURE CANADA. Compendium of animal rabies vaccines marketed in Canada. Can Vet J 1982; 23: 304-305.
2. BROWN AL, MERRY DL, BECKENHAUER WH. One year immunity in dogs vaccinated with high egg passage rabies virus grown on established dog kidney cell line. J Am Vet Med Assoc 1968; 153: 174-179.
3. SIKES RK, PEACOCK GV, ACHA P, ARKO RJ, DIERKS R. Rabies vaccines: duration of immunity study in dogs. J Am Vet Med Assoc 1971; 159: 1491-1499.
4. BROWN AL, MERRY DL, BECKENHAUER WH. Modified live virus rabies vaccine produced from Flury high egg passage virus grown on established canine kidney cell line: three year duration of immunity study in dogs. Am J Vet Res 1973; 34: 1427-1432.
5. STRATING A, BUNN TO, GOFF MT, PHILIPS CE. Efficacy of inactivated tissue culture rabies vaccine in dogs. J Am Vet Med Assoc 1975; 167: 809-812.
6. FIELDS M, AMENT RD, LAMB D, BLADES J. Suckling mouse brain rabies vaccine (SMBV): duration of immunity in dogs. Vet Med Small Anim Clin 1976; 71: 37-40.
7. ZALAN E, WILSON C, PUKITIS D. A microtest for the quantitation of rabies virus neutralizing antibodies. J Biol Stand 1979; 7: 213-220.
8. ABELSETH MK. An attenuated rabies vaccine for domestic animals produced in cell culture. Can Vet J 1964; 5: 279-286.
9. CLARK KA, KELLY VP, NEWMAN EC, BILDERBACK WR, NETTLES WD, RHODES TS. Rabies vaccination. Field observations during epizootics in dogs. Mod Vet Pract 1981; 12: 907-911.
10. ENGELS M, FLUCKIGER M, KNUSLI K, WYLER R. Der immunstatus gegen tollwut bei 200 geimpften hunden aus dem kanton Zurich. Schweiz Arch Tierheilkd 1982; 124: 149-156.

ABSTRACT

GOUGH PM, JORGENSON RD. **Identification of porcine transmissible gastroenteritis virus in house flies (*Musca domestica Linneaus*).** *American Journal of Veterinary Research.* 1983; 44: 2078-2082. (Vet. Med. Res. Inst. Coll. Vet. Med., State Univ., Ames, Iowa 50011, USA).

Transmissible gastroenteritis (TGE) virus was detected in house flies (*M. domestica*) by staining with specific fluorescent antibody. The flies were collected within a swine confinement facility in which TGE was endemic. Laboratory-reared flies were infected experimentally with TGE virus and the virus was recovered from the insects for 72 hours after infection. The TGE virus was identified both by the fluorescent antibody technique and by isolation in cell culture. The nature of plaque formation in cell monolayers inoculated with the virus passaged through flies changed from a large plaque (4 mm or greater in diameter) to a small plaque (1 mm in diameter) over the period. Large plaques were observed early after infection and were attributed to TGE virus mechanically carried by the flies. Small plaques occurred 8 to 12 hours after infection and were considered to be produced by virus replicated in the dipterous cell.

Reprinted from the "Veterinary Bulletin", Volume 54, No. 4, April 1984.