Prevalence of antibodies to four major canine viral diseases in dogs in a Liverpool hospital population

B. J. Tennant*, R. M. Gaskell*, R. C. Jones* and C. J. Gaskell†

Departments of Veterinary Pathology* and Veterinary Clinical Sciences†, University of Liverpool, Liverpool, L69 3BX

Journal of Small Animal Practice (1991) 32, 175-179

ABSTRACT

To determine the prevalence of antibodies to four major canine viruses, serum samples were obtained from 190 dogs presented to the Small Animal Hospital at the University of Liverpool. Antibodies to canine coronavirus (CCV), canine distemper virus (CDV), canine parvovirus (CPV) and rotavirus (RV) were assayed using serum neutralisation (CCV and CDV), haemagglutination inhibition (CPV) and indirect fluorescent antibody (RV) techniques. Overall 54 per cent of dogs were seropositive to CCV, 84 per cent to CDV, 70 per cent to CPV and 86 per cent to RV. The antibody titres obtained were analysed with respect to a number of different parameters including: age, sex, breed, vaccination status, exercise regime, diet, Liverpool district in which the dog resided and the presence of diarrhoea. The prevalence and titres of antibodies to CCV, CDV and RV appeared to be influenced by age, CDV by vaccination status, and CCV by the presence of diarrhoea; no other influencing parameters were found.

INTRODUCTION

Canine enteritis is a common problem in small animal practice and, of the many causes, infectious viral agents are of considerable significance. Canine parvovirus (CPV), first appeared as a cause of severe, often haemorrhagic gastroenteritis with high mortality in 1978 (Appel and others 1978). It has since been shown to have a worldwide distribution, and by early 1980 figures for seroprevalence generally ranged from 25 to 90 per cent (Helfer-Baker and others 1980, Kramer and others 1980, Pollock and Carmichael 1981). More recently Olson and others (1988) in Sweden have reported seroprevalence rates to CPV of approximately 30 to 40 per cent in both unvaccinated and vaccinated adult dogs.

Canine coronavirus (CCV), first isolated from dogs with enteritis in 1971 (Binn and others 1974), is generally thought to cause mild to moderate enteritis. Seroprevalence figures have been reported to range from 4 to 75 per cent in family dogs and 60 to 80 per cent in kennel populations (Cartwright 1973, Pensaert and Callebaut 1978, Helfer-Baker and others 1980, Osterhaus and others 1980, Toma and Moraillon 1980).

Canine distemper virus (CDV) may also cause gastroenteritis in dogs, but other body systems, particularly the upper respiratory tract and CNS are generally also affected (Appel 1987). CDV infection has always been considered to be widespread in dogs (Lauder and others 1954). However, the only recent survey reported seroprevalence rates to CDV in Sweden of approximately 30 per cent in unvaccinated and approximately 70 per cent in vaccinated adult dogs (Olson and others 1988).

Canine rotavirus (CRV), has been implicated as a cause of neonatal canine enteritis since 1980 (England and Poston 1980), and seroprevalence has been reported to range from 62 to 84 per cent (McNulty and others 1978, Dagenais and others 1980).

As most of the published surveys on the seroprevalence of these four canine viruses took place some years ago or in other countries, a prospective survey to assess their current seroprevalence in urban and suburban family-owned British dogs was carried out. For CPV, it was of particular interest to reassess seroprevalence now infection has to a large extent stabilised in the population. For CCV, an assessment of current UK seroprevalence is important because of the possibility that vaccines may be developed in the near future. A number of demographic and other parameters which may influence the prevalence and level of antibody to these viruses were also examined.
MATERIALS AND METHODS

Data collection and analysis

Serum samples were collected from a total of 190 dogs selected from the first-opinion clinic at the University of Liverpool’s Small Animal Hospital over a 10-month period. Serum was obtained from the first three dogs presented for any reason, on each of the first four days each week, for four weeks during each of the months of April, July and October 1985 and January 1986; two samples were unobtainable. Twenty-four of these dogs presented with acute and chronic diarrhoea (15 dogs duration <1 week; five dogs 1 to 3 weeks; four dogs >3 weeks). A questionnaire covering a number of demographic parameters was completed for each dog at the time of sampling. Data requested for each dog included age, breed, sex, Liverpool district (ie suburban or urban) in which the dog resided, vaccination status (ever vaccinated, and up to date vaccination, ie, within the previous two years), diet, whether multidog household and type of exercise. Information was not always obtainable for all parameters in all cases. The influence of these parameters on the presence or absence of serum antibodies to each virus was investigated initially using 2\alpha analysis. Interactive analysis was also carried out using two forms of log-linear modelling. Analysis of deviance (logistic regression) further investigated the influence of parameters on presence or absence of antibody, and regression analysis was used to examine the influence of parameters on actual antibody titres.

Serology

Serum obtained from each dog was heat inactivated at 56°C for 30 minutes and stored in aliquots at -20°C until used.

Virus neutralising (VN) antibodies to CCV (K378 strain) (Barlough and others 1984) and CDV (Onderstepoort strain) were assayed in a microneutralisation test using 100TCID50 of virus and doubling serial dilutions of serum (Gaskell and others 1982). Viruses were grown on A-72 cells (Binn and others 1980) (CCV) or Vero cells (CDV). Haemagglutination inhibition (HI) tests for CPV antibody were performed with 4-8 HA units of a UK CPV field isolate (kindly supplied by Intervet Laboratories), using a standard microtitre system (Carmichael and others 1980), with doubling dilutions of serum. An immunofluorescence (IF) test was used to determine rotavirus antibody titres in a microtitre system using Simian rotavirus SA-11 grown in MA104 cells. Duplicate fourfold serial dilutions of sera were made and inoculated into the wells. After incubation at 37°C for one hour the plates were thoroughly washed with phosphate buffered saline. Fluoroscein labelled rabbit anti-dog IgG (Sigma) was added to each well and plates were further incubated, washed again and examined under an ultraviolet microscope. In some cases serum samples were insufficient for all tests to be performed or were toxic, and some data was therefore unavailable. For rotavirus, only a representative proportion (112) serum samples were assessed.

RESULTS

General demography and diarrhoea vs non-diarrhoea

Of the 190 dogs included in the survey 38 per cent were crossbred and 62 per cent purebred. Fifty-four per cent of the population were male, 29 per cent entire female and 17 per cent spayed female. Twenty-four per cent of dogs came from multidog (ie, two or more dogs) households. Of the 190 dogs 15 per cent were fed home produced food only, 50 per cent fed commercial food only and 35 per cent a mixture of these two diets. Four exercise regimes were investigated: many dogs were exercised using a combination of regimes, 52 per cent of dogs had access to a garden, 73 per cent were taken out on the lead, 68 per cent were also allowed to run free but accompanied and 11 per cent of dogs roamed free. Dogs were evenly distributed throughout the Liverpool area. There were no significant differences by \chi² tests with any of these parameters between dogs with or without diarrhoea.

The age distribution of dogs with and without diarrhoea is shown in Fig 1. Dogs with diarrhoea were significantly younger (P<0.05); 13/24 (54 per cent) of dogs with diarrhoea were under two years of age compared to 25/162 (15 per cent) of
Prevalence of antibodies to four major canine viral diseases

FIG 2. Distribution of antibody titres to (a) canine coronavirus (CCV), (b) canine distemper virus (CDV), (c) canine parvovirus (CPV) and (d) rotavirus (RV). 1 Virus neutralising antibody titre v 100 TCID50 CCV, 2 Virus neutralising antibody titre v 100 TCID50 CDV, 3 Haemagglutination inhibition titre v 4-8 HA units CPV, 4 Immunofluorescence antibody titre v Simian rotavirus

dogs without diarrhoea. In addition, dogs with diarrhoea tended to be non-vaccinated, (7/20 [35 per cent]), compared to those without diarrhoea, (18/132 [14 per cent]) (P<0.05). In spite of the small numbers a model was created for a diarrhoeic dog confirming the findings, which included age and vaccination status (ever vaccinated), but there was no interaction term, ie, both parameters influenced the presence of diarrhoea independently.

Of this population 124/149 (83 per cent) of dogs had been vaccinated to CDV and CPV at some time, of which 101/131 (77 per cent) and 75/122 (62 per cent) of dogs had been vaccinated as puppies for CDV and CPV, respectively. Only 34/140 (24 per cent) and 36/139 (26 per cent) of dogs were up to date with CDV and CPV vaccination, respectively.

Overall antibody estimation

The range of antibody titres for each virus is shown in Figs 2a-d. Serum samples were considered positive to CCV if VN titres were greater than or equal to 1/4 (Keenan and others 1976); to CPV if HI titres were greater than or equal to 1/80 (Carmichael and others 1980); to CDV if VN titres were greater than or equal to 1/20 (Olson and others 1988); and to RV if IFA titres were greater than or equal to 1/16 (Johnson and others 1983). Overall 94/174 (54 per cent) were seropositive to CCV; 146/173 (84 per cent) were seropositive to CDV; 146/173 (84 per cent) were seropositive to CDV; 119/170 (70 per cent) were seropositive to CPV; and 97/112 (86 per cent) were seropositive to RV.

Factors affecting antibody prevalence

On initial x2 analysis age and presence of diarrhoea appeared to influence the presence of CCV antibodies. Significantly fewer young dogs (up to two years of age) were seropositive to CCV as compared to older dogs (P<0.02); the majority of dogs were seropositive from two years of age onwards (Fig 3). Significantly fewer dogs with diarrhoea were seropositive for CCV 6/22 (27 per cent) as compared to 88/152 (58 per cent) without diarrhoea (P<0.01).

The prevalence of antibodies to CDV appeared to be influenced by age and vaccination status (ever vaccinated). Significantly fewer dogs (P<0.02), under the age of six months were seropositive for CDV compared to older dogs (Fig 3). For CDV, a disease for which vaccination is practiced in the UK, vaccination status appeared to significantly influence the prevalence
of antibody to CDV, with 100/119 (89 per cent) of vaccinated dogs seropositive compared to 13/23 (57 per cent) unvaccinated dogs (P<0.01).

The presence of CPV antibodies was not influenced by any factors including age (Fig 3) or vaccination status.

Age appeared to influence the prevalence of antibodies to RV, with dogs under six months of age more likely to be seronegative for RV compared to older dogs (P<0.01) (Fig 3).

For all the other factors considered no differences were shown between seronegative and seropositive populations.

Analysis of deviance, which evens out the influence of each parameter, confirmed the influence of presence of diarrhoea on CCV antibody status, and the effect of vaccination status with respect to CDV antibodies. No significant age effect on the presence of antibody for any of the viruses was shown. Regression analysis showed that levels of antibody titres were also influenced by presence of diarrhoea (CCV) and vaccination status (CDV). No other influences were seen.

**DISCUSSION**

Our findings for overall prevalence of antibodies to these four enteric pathogens of dogs broadly confirm the findings of others for this type of pet dog hospital population, although a higher seroprevalence of CPV in vaccinated and non-vaccinated dogs was found than has generally been reported (Cartwright 1973, Pensaert and Callebaut 1978, McNulty and others 1978, Binn and others 1979, Helfer-Baker and others 1980, Kramer and others 1980, Osterhaus and others 1980, Toma and Moraillon 1980, Olson and others 1988). This may be because CPV infection has become more widespread since the earlier surveys were conducted. Of the various demographic parameters examined, breed, sex, exercise pattern, diet, multidog household and urban or suburban district, had no apparent effect on antibody prevalence.

χ² analysis suggested that age may influence the presence of antibody to CCV, CDV and RV, in that younger dogs were less likely to be seropositive. This finding was not confirmed by interactive analysis, but this may be due to the small sizes of the subgroups generated. The age at which seroconversion occurs depends for all four viruses upon the time of exposure to field virus and, for CDV and CPV alone, on time of vaccination. For CPV, the majority of dogs had antibody by six months of age, in contrast to CDV and RV where most dogs appear to encounter the virus, as demonstrated by seroconversion, after six months of age. For CCV seroconversion appears to take place more gradually over a period of several years. This may reflect the age at which field challenge tends to occur. It is interesting that in our study most dogs did not seroconvert to RV until over six months of age. In most species rotavirus infection generally seems to be associated with neonatal animals (Woode and Bridger 1975, McNulty and others 1976, Snodgrass and others 1976, England and Poston 1980). This may be a reflection of the different environments in which different species are reared.

Several factors appeared to be important with respect to diarrhoea. Of the dogs with diarrhoea, only 27 per cent were seropositive to CCV compared to 58 per cent of the non-diarrhoea group suggesting that this group of dogs were susceptible to CCV at the time of clinical presentation and may have been undergoing acute CCV infection. However, diarrhoea mostly occurred in younger dogs whereas the highest seroprevalence to CCV was in older animals. Alternatively, other agents may have been responsible for the diarrhoea. Thus the higher prevalence of diarrhoea in unvaccinated animals might suggest involvement of CPV or CDV. Convalescent sera demonstrating rising titres would have enabled confirmation of these hypotheses, but in this type of first opinion, subsidised clinic such samples were unavailable. Parallel virus isolation and electron microscopic examination were carried out on a number of faecal samples (Tennant 1989) and CPV was isolated from 1/24 dogs with diarrhoea and from none of the control dogs; with CCV isolation studies faecal samples were unfortunately subject to storage problems. Thus we were unable to determine the aetiology of the diarrhoea seen.

Both the presence of and level of antibody titre to CDV were influenced by whether the dog had ever been vaccinated or not. Significantly more dogs vaccinated at some time to CDV had antibody compared to those that had not and similar findings were reported by Olson and others (1988) in Sweden. It appears that CDV vaccination, rather than widespread infection, plays an
important role in inducing a significant level of seropositivity to CDV within a population. Also, vaccination, but not up to date, influenced the presence of CDV antibodies supporting the suggestion that CDV antibodies may persist for several years (Krakowka and others 1985, Olson and others 1988).

In contrast to CDV, no factors, including vaccination status, were found to influence the presence or level of CPV antibodies. Olson and others (1988) reported similar findings in a Swedish canine population, where no difference in CPV titres was obtained between vaccinated and unvaccinated adult dogs. The comparable prevalence of CPV antibodies in all age groups suggests an early exposure of young dogs to CPV and continuous boosting of antibody levels by natural challenge, thus masking any vaccine-derived antibody. It is recognised that CPV antibodies arising from natural infection may persist at high levels for several years (Appel 1987).

ACKNOWLEDGEMENTS

We would like to thank Dr Frank Scott, More-dum Research Institute, both for advice with the rotavirus work and for kindly supplying the rotavirus infected plates. Dr R. M. Gaskell was supported by the Whitley Animal Protection Trust and Dr B. Tennant by Intervet Laboratories.

REFERENCES

APPÉL, M. J. (1987) Canine distemper virus. In Virus Infections of Carnivores. Ed M. J. Appel. Elsevier Science Publishers, New York.

APPÉL, M. J., COOPER, B. J., GREEN, H. & CARMICHAEL, L. E. (1978) Status report: canine viral enteritis. Journal of the American Veterinary Medical Association 181, 1510-1518

BARLOUGES, J. D. (1985) A rotaviruses infection of the canine enteritis. Journal of Veterinary Record 117, 486-487

BRODIE, R. J., BRADLEY, D. & WALL, H. G. (1979) Studies of respiratory disease in random-source laboratory dogs: viral infections in unconditioned dogs. Laboratory Animal Science 29, 48-42

BROEMER, R. N., LASS, C., KEENAN, K. P., HUXSOLL, D. L., CARMICHAEL, L. E., CROW, L. M., BRATTON, R. D., WALL, H. G. (1978) Establishment of a canine cell line: derivation, characterisation and viral spectrum. American Journal of Veterinary Research 41, 855-860

CARMICHAEL, L. E., JOUBERT, J. C. & POLLOCK, R. V. H. (1980) Establishment of a canine cell line: derivation, characterisation and viral spectrum. American Journal of Veterinary Research 41, 855-860

CARTWRIGHT, S. (1973) A vomiting and diarrhea syndrome in dogs. Veterinary Annual 14, 199-195

DAGENais, L., SCHWEIS, A., LANSIVAL, B. & PASTORE, P. P. (1980) Recherches d'anticorps dirigés contre le rotavirus bovin dans le serum des chiens, chats et chevaux en Belgique. Annales de Medicine Veterinaire 124, 423-428

DIXON, J. J. & POSTON, R. P. (1980) Electron microscopic identification and subsequent isolation of a rotavirus from a dog with fatal neonatal diarrhoea. American Journal of Veterinary Research 40, 782-783

GASKELL, R. M. & GASKELL, C. J. (1985) EFFICACY OF AN INACTIVATED FELINE CALICIVIRUS (FCV) VACCINE AGAINST CHALLENGE WITH UNITED KINGDOM FIELD STRAINS AND ITS INTERACTION WITH THE FCV CARRIER STATE. Research in Veterinary Science 32, 23-26

HELFER-BAKER, C., EYERMANN, J. E., McGRENNN, A. J., MURPHY, W. B., SLACK, R. L. & MILLER, C. W. (1980) Serological studies on the incidence of canine enteritis viruses. Canine Practice 3, 37-42

JOHNSON, C. A., FULTON, W., HENK, W. G. & SNIDER, T. G. (1983) Incubation of neonatal gnotobiotic dogs with a canine rotavirus. American Journal of Veterinary Research 44, 1682-1686

KEENAN, K. P., JERVIS, H. R., MARCHWICKI, B. S. (1973) A characterisation of a coronavirus from military dogs with diarrhoea. Proceedings of the 78th Meeting U.S. Animal Health Association, 359-366

KRAMER, J. M., MEUNIER, P. C. & POLLOCK, R. V. H. (1980) Canine parvovirus: update. Veterinary Medicine and Small Animal Clinician 75, 1541-1555

LAUDER, I. M., MARTIN, W. B., GORDON, E. B., LAWSON, D. D., CAMPBELL, R. S. & WATRACH, A. M. (1954) A survey of canine distemper. Veterinary Record 65, 607-611

McNULTY, M. S., ALLAN, G. M. & MCFERRAN, J. J. (1976) Isolation of a cytopathic calf rotavirus. Research in Veterinary Science 21, 114-115

McNULTY, M. S., ALLAN, G. M., THOMPSON, D. J. & O'BOYLE, J. D. (1976) Antibody to rotavirus in dogs and cats. Veterinary Record 102, 534-535

OLDENBURGH, U., DANNER, K. & KRAUSS, H. (1964) Zur betzung von rotavirus-infektionen beim hund. Zentralblatt Veterinaermedizin B 31, 297-302

OLSON, P. K., KLEINBERG, B. & HEIDHAMMER, A. (1988) Serum antibody response to canine parovirus, canine adenovirus-1 and canine distemper virus in dogs with known status of immunization: study of dogs in Sweden. American Journal of Veterinary Research 49, 1460-1466

OSTERHUS, A. D. M. E., DROST, A., WIRAHADRISELJA, R. M. S., VAN DEN ENGH, T. S. G. A. M. (1980) Canine viral enteritis: Prevalence of parvo-, corona- and rotavirus infections in dogs in the Netherlands. Veterinary Quarterly 2, 181-189

PENSAERT, M. & CALLEBAERT, P. (1978) The coronaviruses: Clinical and structural aspects with some practical implications. Annales de Medicine Veterinaire 122, 301-322

POLLOCK, R. V. H. & CARMICHAEL, L. E. (1981) Newer knowledge about canine parovirus. In Proceedings 30th Gaines Veterinary Symposium, 36-40

SNOOGRASS, D. R., SMITH, W., GRAY, E. W. & HERRING, J. A. (1976) A rotavirus in lambs with diarrhoea. Research in Veterinary Science 20, 113-114

TENNANT, B. J. (1989) Studies on the epizootiology and pathogenesis of canine coronavirus infection in the dog. PhD Thesis, University of Liverpool

TOMA, B. & MORAILLON, A. (1980) Infection du chien par un virus antigeniquement apparente au virus de la gastroenterite transmissible du porc. Recueil de Medicine Veterinaire 156, 464-470

WOODS, G. & BRIDGER, J. (1975) Viral enteritis of calves. Veterinary Record 96, 85-88

Prevalence of antibodies to four major canine viral diseases