Canine coronavirus in Australian dogs

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Objective To estimate the frequency of serum antibodies (IgG and IgM) to canine coronavirus (CCV) in the Australian dog population and evaluate the role of CCV as a causative agent of gastroenteritis.

Design A serological survey of antibodies to CCV among different dog populations.

Procedure The development and characterisation of an indirect ELISA for the detection of antibodies (IgG and IgM) to CCV was undertaken. Sera collected from both diarrhoeal and non-diarrhoeal dogs from various populations throughout Australia were tested for these antibodies to CCV.

Results Serum samples (1396) collected from 1984 to 1998 were tested for the presence of IgG antibodies to CCV. Samples were divided into two categories on the basis of the number of dogs housed together. The groups were either an open population containing dogs housed as groups of three or less, or kennel populations. Sera from 15.8% of the open population and 40.8% of kennelled dogs were positive for CCV antibodies. The prevalence of antibodies varied from zero to 76% in kennelled dogs. About 23% of 128 dogs positive for IgG antibodies to CCV were also positive for IgM antibodies to CCV, indicating recent CCV infection. Of those dogs that were presented with clinical signs of gastroenteritis such as diarrhoea and vomiting (n = 29), 85% were positive in the IgM ELISA and 85.7% in the IgG ELISA for antibodies to CCV. In comparison, for those dogs presented without any history of gastroenteritis only 15% were positive for IgM and 30% positive for IgG.

Conclusion Serological evidence indicates that infection with CCV in dogs is widespread throughout the Australian mainland. The prevalence of antibodies varies greatly among different populations, with an average of 40.8% positive in kennelled populations and 15.8% in the open population.

Key words: Dogs, canine coronavirus, gastroenteritis, ELISA, serological survey.
was ultracentrifuged for 30 min at 90,000 g (4°C) and the supernatant used as antigen for the ELISA. Control antigen was prepared in the same manner from uninfected cells.

Indirect ELISA methods

Indirect ELISAs for the detection of serum IgG or IgM antibodies to CCV were developed on the basis of previously published assay methods.10,11 Control and CCV TN-449 antigens were diluted separately to 2 μg per 0.1 mL in carbonate-bicarbonate buffer and incubated overnight (4°C) in 96-well flat-bottomed Immunoplates (Nunc, Denmark) with 0.1 mL per well of diluted antigen. The solution was decanted from the plates and 0.1 mL per well of 0.1% skim milk in PBS-blocking agent was added. After incubation for 1 h at 37°C, plates were washed once with PBS containing 0.05% Tween 20. Positive and negative control sera and test sera were serially diluted in PBS containing 0.05% Tween 20 with 10% foetal bovine serum (CSL Biologics, Australia), inactivated at 57°C for 1 h and added at a volume of 50 μL per well. Dilutions were incubated for a further hour at room temperature before the plates were decanted and washed three times with PBS containing 0.05% Tween 20. Fifty μL per well of a 1:6000 dilution of either HRP-conjugated rabbit anti-dog IgG (Sigma, Australia) or HRP-conjugated goat anti-dog IgM (Nordic, The Netherlands) was added and the plates incubated for 1 h at room temperature. Following a further three washes, 100 μL per well of 0.1 mg per mL tetramethylbenzidine microwell peroxidase substrate (Kirkegaard & Perry Laboratory, USA) was added and incubated for 10 min. One hundred μL of 0.3M phosphoric acid was added to stop the reaction and the absorbance was read at 450 nm. Each serum dilution was performed in duplicate, with the absorbance value of the control antigen deducted from the CCV TN-449 antigen absorbance value to give the final value. An absorbance value greater than 0.1 was considered a positive reaction. This value was at least two times higher than background values from negative control sera (unpublished data).

Population

Serum samples (n = 1396) were collected from dogs in two different housing groups. One group contained dogs housed in groups of no more than three animals (open population) while the other group contained dogs from commercial breeders, rescue shelters and remote settlement colonies where dogs were housed as a group of 12 or more animals (kennel populations) (Table 1).

Open population - In this group, 1107 serum samples were collected from veterinary pathology laboratories, universities and by field sampling from dogs submitted for general pathology, surgery or survey purposes. These dogs were domestic pets from both suburban and rural areas, generally kept singly or in pairs. The sample areas included all the Australian states and territories, with sera collected from 1984 to 1998.

Kennen populations - In this group, 289 serum samples were collected from three commercial breeders, a university study, three rescue kennels and four remote settlement colonies.

Serum samples

All serum samples were stored at -30°C before use. All samples were tested for IgG antibodies and 128 randomly selected IgG positive sera were tested for IgM antibodies to CCV. Fourteen IgG-negative serum samples derived from dogs at sites where episodes of gastroenteric infection had recently occurred, were also tested for IgM antibodies to CCV.

Statistical analysis was carried out using unpaired t-tests (Statview version 4.5; Abacus Concepts Inc, USA).

Table 1. Detection by ELISA of IgG antibodies to canine coronavirus in Australian dog populations.

| Housing             | No. tested | No. positivea | % positive |
|---------------------|------------|---------------|------------|
| Open population     |            |               |            |
| New South Wales     | 276        | 66            | 23.9       |
| Northern Territory  | 20         | 0             | 0          |
| South Australia     | 91         | 7             | 7.7        |
| Queensland          | 266        | 73            | 27.4       |
| Western Australia   | 200        | 13            | 6.5        |
| Victoria            | 109        | 6             | 5.5        |
| Tasmania            | 1          | 1             | 100        |
| Mixed (Australia)b  | 144        | 9             | 6.3        |
| Total               | 1107       | 175           | 15.8       |
| Kennel populations  |            |               |            |
| Breeding colony 1   | 33         | 25            | 75.8       |
| Breeding colony 2   | 30         | 14            | 46.7       |
| Breeding colony 3   | 24         | 18            | 75.0       |
| University study    | 44         | 13            | 29.5       |
| Rescue shelter 1    | 16         | 3             | 18.8       |
| Rescue shelter 2    | 23         | 9             | 39.1       |
| Rescue shelter 3    | 25         | 18            | 72.0       |
| Remote settlement colony 1 | 35     | 15 | 42.9 |
| Remote settlement colony 2 | 29 | 2 | 6.9 |
| Remote settlement colony 3 | 18 | 0 | 0 |
| Remote settlement colony 4 | 12 | 1 | 8.3 |
| Total               | 289        | 118           | 40.8       |
| Grand total         | 1396       | 293           | 21.0       |

aAn absorbance value of greater than 0.1 (OD, 450 nm) was considered a positive reaction.

bState not recorded
value for a positive result) to 0.590. For the open population group the majority (81%) of readings were in the range 0.100 to 0.199. In comparison 58.5% of values for the kennel populations were greater than an OD of 0.199.

IgM antibody to CCV

Twenty-nine of the 128 (22.7%) randomly selected, IgG antibody-positive sera were also found to be positive for IgM antibodies to CCV. Clinical histories were available for 20 of these 29 dogs and 17 (85%) had clinical signs of diarrhoea. Among the IgG negative sera, fourteen samples were also tested for IgM antibodies to CCV. These samples were selected on the basis of a high prevalence of gastroenteric disease in the kennelled population at the time of sampling (Breeding colony 1, N SW, 8 samples) or evidence from clinical records of a recent episode of gastroenteritis in the open population dogs sampled (6 samples). Seven (50%) of these dogs were positive for anti-CCV IgM antibodies.

Discussion

The prevalence of CCV antibodies in different dog populations throughout the world has been found to range from 6 to 75%, with as high as 80% reported in kennelled populations. We report the first serological study of canine coronavirus antibodies in the Australian dog population. The prevalence of CCV antibody was 15.8% (0 to 27.4%) in dogs housed singly or in small groups (open population) but a significantly higher (P < 0.0001) prevalence of 40.8% (0 to 76%) was found among kennelled dogs. CCV has been reported previously in Australia based on electron microscopic examination of faeces; these two studies showed 7.1% of 154 dogs positive, and 2.9% of 102 dogs positive. In the open population of 1107 dogs tested we found 15.8% positive for anti-CCV IgG antibody, which reflects past exposure and infection with CCV whereas the electron microscopic studies detected only those dogs currently infected and shedding virus in their faeces. Dogs positive for CCV antibody were found in every Australian state and territory, including remote areas of the Northern Territory. Schnagl and Holmes had also previously reported CVLP in remote areas of Western Australia and Northern Territory. One dog with antibodies to CCV was found in Tasmania, although no other sera were tested from that state. We conclude that CCV exists in dogs throughout Australia.

In the open populations, most ELISA results fell within an OD range of 0.1 to 0.199 (unpublished data). Within the kennel populations, approximately 60% of OD readings were greater than 0.199 (P > 0.0001). The difference in the occurrence of exposure among dogs from kennel populations and the open population has been observed previously. The higher frequency of exposure found in kennel populations presumably represents the increased opportunity for exposure due to the different housing and social interactions.

In the three commercial breeding colonies from NSW examined in this study, severe gastroenteric disease resulting in variable fatality among young pups had been previously observed (M. Lindsey personal communication). In these cases CPV was reported as the causative agent. The current study identified high titres of CCV antibody in these kennels during the same period. IgM antibodies to CCV were also detected in these populations, indicating current CCV infection at the time of the gastroenteritis epidemics. It is probable that a mixed infection of CCV and CPV was occurring and the presence of CCV may have contributed to the severity of the enteritis. Appel and Brunner and Swango demonstrated increased severity of clinical signs of both CCV and CPV in mixed infections, particularly in young and stressed animals. It is therefore difficult to assess the clinical implications of CCV infection alone, because mixed infections or other factors may influence the course of disease.

The 24 dogs that were found to be positive for CCV antibody and had signs of gastroenteritis, may represent examples of CCV induced disease as these dogs were also negative for many of the other common gastroenteric pathogens such as CPV (unpublished data). A highly significant correlation (P < 0.0001) was found between the presence of anti-CCV IgG antibodies and diarrhoea, with 24 of 28 dogs (85.7%) that presented with diarrhoea being IgG positive compared with 40 of 134 (30%) dogs that presented without diarrhoea. Approximately 23% of 128 dogs positive for IgG antibodies to CCV were also positive for IgM antibodies to CCV indicating infection within 14 days of blood samples being collected. Seven dogs were identified as IgG antibody negative and IgM antibody positive to CCV. These dogs represent examples of animals exposed to CCV within 2 to 5 days before blood samples were collected and that had not had time to produce a detectable IgG antibody response. All seven of these dogs had signs of gastroenteritis. Eighty-five percent of dogs that had clinical signs of gastroenteritis attributed to CCV infection were positive for IgG and IgM antibody to CCV. In comparison, for those dogs presented without any history of gastroenteritis only 15% were anti-CCV IgM positive (P < 0.0001). Our study indicates that CCV is widespread in the Australian dog population and suggests that the presence of CCV antibodies is associated with gastroenteritis.

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OBITUARY
Marjorie Ann Reid

Marjorie Reid was a remarkable woman who epitomised the wealth and breadth of veterinary training, by successfully accepting challenges that ranged from practice, laboratory, fieldwork and senior veterinary administration. Marjorie, who was born in 1926, graduated from the Royal Veterinary College in 1950 and was one of a small, but elite group of women in the veterinary profession in England. She had always had a strong sense of adventure and pioneering spirit and, in 1951, she commenced practice in South Africa. Later, she moved to Kenya and joined the well known Veterinary Research Institute at Kiboko near Nairobi. Marjorie enjoyed working in Kenya, where there was a constant challenge to use basic veterinary principles to provide a diagnostic service for commercial and wild animals. Her two daughters were born during this period in Kenya. During 1963 she immigrated to Australia and became the first female veterinarian at the Institute of Medical and Veterinary Science in Adelaide. It was during this period she became interested in bacteriology and began to develop her skills in this subject.

In 1966 she joined the Department of Agriculture, Stock and Fisheries in Papua New Guinea and was based at the Kila Kila Veterinary Laboratory in Port Moresby. When she arrived the laboratory was a converted airport control tower from the Second World War. This tested her strengths in more ways than one, not only in bacteriology, but also filling in as Port Moresby's veterinarian on occasions. With her colleagues, Ilor Owen and Noel Talbot, she showed a remarkable ability to use good practical commonsense to simplify what seemed to be highly complex issues. When the new laboratory was built, she was able to demonstrate her considerable laboratory skills to all and was a good but firm teacher to many. This was a happy and productive period of her life. Marjorie was very good with animals of all species ranging from the usual to exotic species such as crocodiles. Because of her combination of ability and character, she was the ‘preferred vet’ in a strongly male-dominated society, which reflected her high standing in the community. Many at first underestimated her strength of character and discipline; everyone soon learnt not to loaf about when Marjorie was around.

In 1974, Marjorie was invited back to South Australia to organise the Brucellosis and Tuberculosis Eradication Scheme in cattle. Again true to form, she was the first woman to join the Department of Agriculture in South Australia. Due to her efforts and management skills she orchestrated a successful Eradication Scheme. The effort to coordinate all aspects of the Brucellosis campaign, with limited resources in a big state, was a difficult task. Marjorie was appointed Principal Veterinary Officer in 1976 and Deputy Chief Veterinary Officer for South Australia in 1986.

She retired in 1990 to live in the Adelaide Hills, devoting some of her time on a voluntary basis to the Guide Dogs for the Blind. Marjorie was the first woman in many of her appointments and she was able to achieve this due to her strong character, abundant wit and sense of humour. She enjoyed a good argument or discussion and, with her formidable memory for dates and wide reading, she was not an easy person to get the better of. Those who tried, felt the sharpness of her wit. An agitated Marjorie was a sight to behold. There are still anecdotes circulating around PNG about Marjorie, when someone accidentally locked her in the cold room.

In 1998 she moved to Sydney to be closer to her family and died on 6 June 2000, after a long illness during which she showed her characteristic strength of character. The veterinary profession is very proud of Marjorie Reid’s contribution to the welfare of animals in Australia and internationally.

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