

**Acute Canine Infectious Tracheobronchitis (kennel cough)  
in Greyhounds and other dogs in New Zealand**

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## Introduction

Canine infectious tracheobronchitis is a term used to describe an acute, highly contagious respiratory disease that affects the larynx, trachea, bronchi, and occasionally the lower respiratory tract and parenchyma. The term "kennel cough" is the commonly used synonym, although Acute Canine Infectious Tracheobronchitis (ACIT) is used here. Clinical signs range from very mild to severe, and reflect the localisation of infection to the respiratory mucosa. When mild, the dominant clinical sign is a dry sounding cough, but which is often associated with expectoration and gagging, and recovery usually occurs within 5-14 days. More severe forms have been reported as sporadic complicated cases, and as outbreaks of more serious disease. Secondary bacterial bronchopneumonia may develop in severely affected or immunocompromised dogs, and acute severe haemorrhagic forms have been described that may be associated with one or two single pathogens.

Although "kennel cough" is a single term, it is used to cover acute infections caused by several different organisms (pathogens). *Bordetella bronchiseptica*, canine parainfluenza virus (PI) and canine adenovirus type-2 (CAV-2) are traditionally considered as the principal pathogens responsible for kennel cough. Vaccination against these pathogens is widely practiced, and the term "kennel cough vaccine" is commonly used. Sadly, the term is misleading, and it is unfortunate that it exists, since it creates the impression that once vaccinated, a dog is protected against infection. However, it is not uncommon for vaccinated dogs to develop clinical signs of kennel cough, which can be due to vaccine failure, infection with a novel strain of the organisms in the vaccine, or involvement of new pathogens that have are not included in the currently available vaccines. Since 2012, we have been notified of 6 outbreaks of kennel cough in racing greyhounds that have resulted in disruptions in racing schedules, and illness in dogs. It is likely that there have been other episodes as well. These have occurred despite the widespread vaccination.

Pathogens other than the traditionally considered "principle pathogens" include bacteria that that can cause primary infections, or act as secondary "opportunistic" pathogens, and include *Pasturella multocida*, and *Streptococcus zooepidemicus*. Viruses other than PI and CAV-2 include the canine respiratory corona virus (CRCoV), canine herpes virus, and canine influenza. Neither canine influenza nor CRCoV have yet been isolated in New Zealand, although there is evidence from an antibody study that CRCoV is present here.<sup>1</sup>

In 2012, it was decided to attempt to identify the causative agents in kennel cough in New Zealand Racing Greyhounds, for the following reasons:

- To determine the relative importance of CAV-2, *Bordetella bronchiseptica* and parainfluenza in decision making regarding vaccination
- To understand the risk of infection with pathogens not covered by the currently available vaccines

- To determine the need for new vaccines in NZ (e.g. CRCoV)
- To remain vigilant for new and potentially more pathogenic respiratory pathogens such as canine influenza.

The initial objectives of this study were:

1. Survey the seroprevalence and shedding of candidate viral, bacterial, and mycoplasma organisms in clinically unaffected dogs in NZ
2. To isolate and identify the specific pathogens present in clinical cases of ACIT over an extended period

Since commencement, we have refined these objectives to include:

3. To evaluate the seroprevalence of CoCRV in New Zealand dogs, and to determine if there is a difference between pet dogs and Racing Greyhounds
4. To evaluate for seasonal variations in CoCRV serological responses in NZ dogs
5. To isolate, by cell culture techniques, the CoCRV present in NZ dogs, and determine the genetic sequence of the NZ isolate to determine its similarity to isolates in other countries
6. To look for hitherto unknown or unidentified pathogens in affected dogs

## **Materials and Methods**

### ***Virological and bacterial survey of unaffected dogs***

#### *Normal Dog recruitment*

- a) Racing Greyhounds. Samples have been collected on a convenience basis by predominantly NZGRA-affiliated veterinarians from dogs belonging to consenting owners. Inclusion criteria were; no clinical history of respiratory disease or evidence of respiratory disease as evidenced by cough, sneeze, nasal discharge, abnormal breathing. Dogs were excluded if they had been vaccinated within 4 weeks of presentation.
- b) Pet dogs. Dogs have been recruited from participating practices in the North and South Islands. Inclusion and exclusion criteria were the same as for Greyhounds. The majority of dogs recruited were anaesthetised for dental prophylaxis or other surgery.

#### *Swab collection*

Swabs were collected from the tonsillar region of the oropharynx. Initially, one swab was submitted for microbial culture, and one submitted for viral isolation and PCR (viral and mycoplasma). However, subsequent swabs were subjected to viral isolation and PCR techniques only.

Venous blood (5-10mL) was collected from the cephalic or jugular vein and allowed to clot at ambient temperature. Serum was harvested and stored at -18°C pending serological analysis.

### ***Investigation of clinical cases***

#### *Clinical disease recruitment*

We have attempted to sample dogs that have recently developed clinical signs of ACIT. Notices have been posted in the Companion Animal Society newsletter, and VetScript. In addition, notices have been issued through the veterinary network of the NZGRA. Veterinarians have been asked to submit samples from any affected dog. We have asked for oropharyngeal swabs, and when possible, for serum samples collected at the initial visit, and 3-4 weeks later, to test for a rise in antibodies following infection. The hope was to investigate isolated cases and clusters or outbreaks.

Upon contact being made from veterinarians, sampling packs were couriered to the practice that day, with provision for return by courier. Serum was frozen, and swabs were processed for viral isolation and DNA/RNA extraction.

## ***National seroprevalence of canine respiratory corona virus***

### ***Sample recruitment and testing***

Serum samples were obtained from the New Zealand Veterinary Pathology diagnostic laboratory. The sample population consisted of dogs sampled for a wide range of reasons, including annual health checks, pre-anaesthetic checks, routine tests, as well as for diagnostic reasons in diseased dogs. Samples were submitted from all regions of the country. Each month, 100 serum samples were selected from the list of submitted samples. Samples were obtained for 10/12 months across 1 year (2014-2015). The only inclusion criteria was the availability of sufficient serum of a suitable quality. However, any sample from a greyhound was positively selected for. Samples were otherwise randomly selected. All samples were tested for CRCoV antibodies using the same inhibition ELISA, and factors such as month, region, and breed were tested for significant effects.

### ***Assays***

- Bacteriological culture of oropharyngeal swabs, including selective media for *Bordetella bronchiseptica*, was performed using conventional isolation and identification techniques. Any  $\beta$ -haemolytic streptococci were further speciated.
- Assay optimisation, and investigation into the influence of sample collection and handling into diagnostic sensitivity of *Bordetella bronchiseptica* was made
- Swabs were suspended in transport media, which was divided into two samples:
  - o Virus isolation: media from samples were incubated with 2 different cell culture systems and observed for viral cytopathologic effects
  - o Multiplex quantitative PCR: DNA and RNA were extracted and submitted to rtPCR or standard PCR for the detection of: parainfluenza, respiratory corona virus, adenovirus-2, canine herpes virus, canine influenza, canine distemper virus, *Strep zooepidemicus*, and *Mycoplasma cynos*. This was conducted at a diagnostic laboratory in Australia (IDEXX)
  - o Subsequent to concerns about the lack of sensitivity using the IDEXX system, we developed and optimised a PCR system to detect: Parainfluenza, CAV-2, and Canine Herpes virus.
- Serum IgG to CRCoV was detected using an inhibition ELISA.

### ***Analysis***

Data analysis has largely been descriptive with the intent of determining the prevalence of the pathogens in the unaffected population, and their relative importance as causes of clinical disease. In addition, we have tested for geographical, seasonal, and breed-specific differences in CRCoV antibody titres.

## Results

### ***Sample collection***

#### Swabs

Normal dog: Swabs have been collected from 73 normal, apparently healthy dogs, of which we also have CRCoV serology in 59. Of the 73, 13 were racing greyhounds.

Diseased dogs: We have collected swabs from 145 clinically affected dogs. Of those, 102 samples have been subjected to quantitative PCR, and another 43 to viral isolation. Extracted genetic material from almost all diseased dogs' samples is currently still being held for further analysis (see below). A total of 50 diseased racing greyhounds have been sampled. Of those, 42 have been subjected to multiplex quantitative PCR.

#### Serum

We have performed CrCoV serology on 49 dogs with ACIT, of which 40 were greyhounds. In addition, we have performed serology on almost 1000 samples obtained from NZVP.

#### 1. Bacterial pathogens

The key bacterial pathogens considered were *Bordetella bronchiseptica*, and *Streptococcus zooepidemicus*. *Bordetella bronchiseptica* is a fastidious organism that is difficult to routinely isolate. Using a reference strain of the bacteria, we have found that the swab material influences the sensitivity of culture and detection by PCR as well as the transport media. *B. bronchiseptica* may multiply in Amies transport media with charcoal and more organisms can be isolated after initial incubation for 48 hr at room temperature. The room temperature helps to maintain the viability of *B. bronchiseptica*, and reduces other bacterial species. Using PCR for detection, we have shown that transport media can inhibit the reaction and reduce PCR sensitivity. We have also found that the sensitivity of PCR performed directly on the nasal swab is influenced by the microbial complexity of the sample. In a mixed infection there is a possibility of bacterial overgrowth leading to few viable *B. bronchiseptica* in the transport media. Thus it is possible that isolation of *B. bronchiseptica* from clinical cases is still underestimated.

Despite combining improved isolation techniques with the molecular detection method (PCR) which does not require collection of live bacteria, we have not found either bacteria to be common in either healthy or diseased dogs. Of the initial 103 diseased dogs tested, *Bordetella* was found in only 4 (3.8%), and *Strep zooepidemicus* was not detected in any. For those reasons, we elected to discontinue attempts to isolate bacterial pathogens.

## 2. *Mycoplasma cynos*

*Mycoplasma* was detected by PCR in 10/103 (9.7%) diseased dogs. This included 5 that were obtained from the same outbreak in Christchurch (Shirley Veterinary Clinic), and were submitted together. We investigated the possibility of studying *M.cynos* further, however we elected to discontinue that because of the lack of a reference organism in NZ for use as a "control" in studies, our inability to obtain permission, the technical difficulty in culturing the organism, and its apparent unimportance in the initial 103 diseased dogs.

## 3. *Viruses*

### Quantitative PCR

In the initial 103 diseased dogs (including 42 greyhounds), the following quantitative PCR results were obtained:

<b>Pathogen</b>	<b>Number qPCR positive</b>
<b>Canine Parainfluenza</b>	3
<b>Canine respiratory corona virus</b>	0
<b>Canine Herpes virus 1</b>	1
<b>Canine Adenovirus-2</b>	5

Some dogs were shedding more than one organism, thus, in total, for all the 103 diseased dogs initially evaluated by qPCR, a known respiratory pathogen was detected in only 19 dogs (18.4%). We consulted with the testing laboratory but were unable to obtain adequate explanation of their protocols to determine if the techniques employed would detect the organisms reliably, and what the sensitivity of their assays are.

### In-house PCR

Of the original 103 samples, we tested 49 from which we had sufficient extracted DNA, for three of the viruses. Of those 49 samples, we obtained the following results:

<b>Pathogen</b>	<b>Number PCR positive</b>
<b>Canine Parainfluenza</b>	3
<b>Canine Herpes virus 1</b>	0
<b>Canine Adenovirus-2</b>	34

It was concluded from the in-house PCR that:

a) Many dogs shed CAV-2. This is almost certainly from previous vaccination, rather than as a cause of their respiratory disease

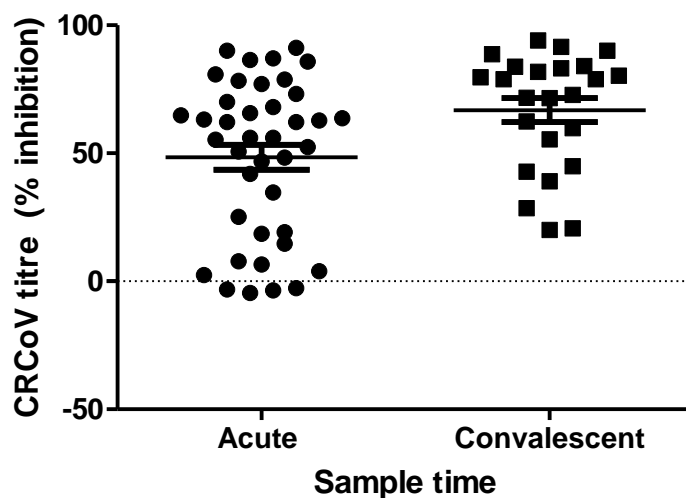
b) The IDEXX multiplex qPCR system appears insensitive. This conclusion would be consistent with the low number of positives found from the 103 swabs collected from affected dogs.

In responses to this disappointing finding, we have discontinued testing samples using the IDEXX system, and have kept subsequent samples for future, more sensitive molecular techniques.

#### Canine respiratory corona virus serology

A previous small serosurvey in NZ suggested that CRCoV might be common in NZ.<sup>1</sup> When combined with our concerns of the inaccuracy of the IDEXX qPCR data, we decided to test for antibody responses to CRCoV in affected greyhounds, other dogs, and then more widely using the NZVP sample set.

Acute vs. convalescent antibody responses to CRCoV in affected greyhounds. 40 greyhounds with ACIT had blood collected at the time of presentation (acute) and 24 of those had samples collected 3-4 weeks later (convalescent). It was found that there was a large number of dogs that had positive antibody tests on the acute sample, but that there was a significant increase in the 24 dogs tested 3-4 weeks later (See figure 1 below).

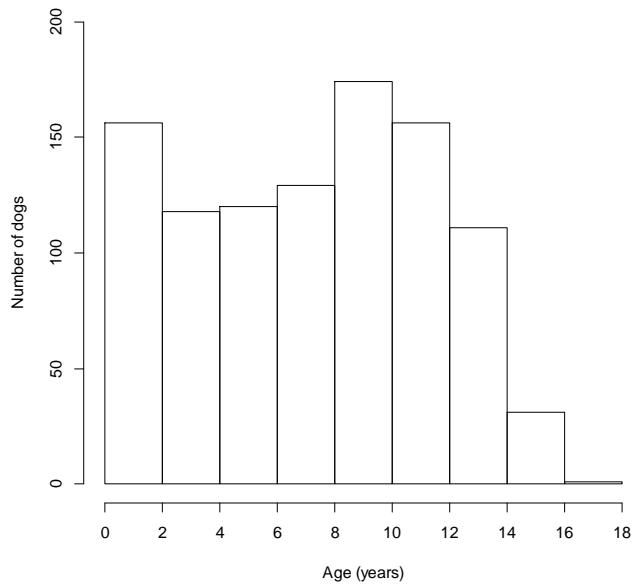


**Figure 1.** Serum antibody to CRCoV in 40 greyhounds with ACIT. The increase in antibody in 24 of the Greyhounds tested 3-4 weeks later was highly significant ( $P=0.004$ ).

The conclusion from this finding is that CRCoV exposure is common amongst greyhounds in NZ, and that it might be responsible for a large percentage of cases of ACIT.

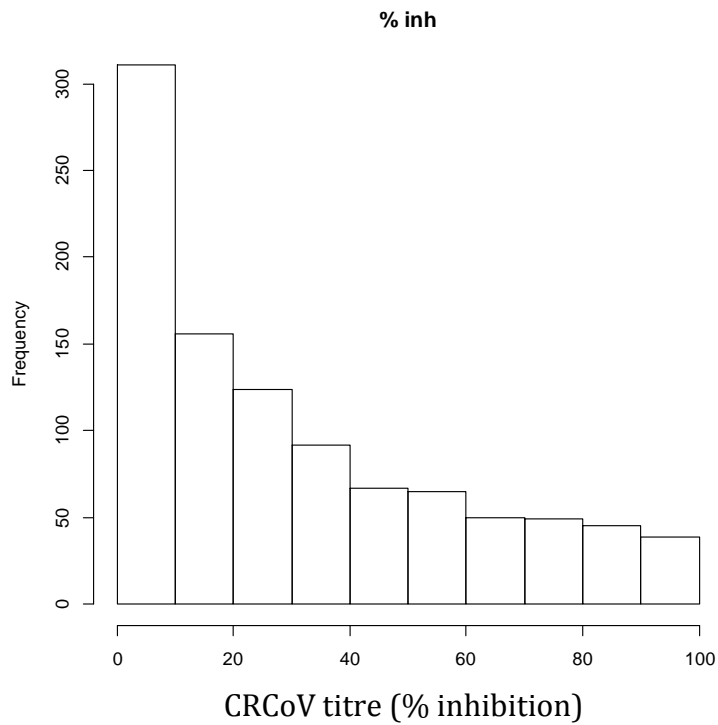


To understand the role of CRCoV in NZ better, we have continued to test affected dogs, and conducted the survey of a wide range of dogs, using the NZVP sample set. The age distribution of the 999 tested dogs is displayed in figure 2.



**Figure 2.** Age distribution of 999 randomly selected dogs for CRCoV antibody testing.

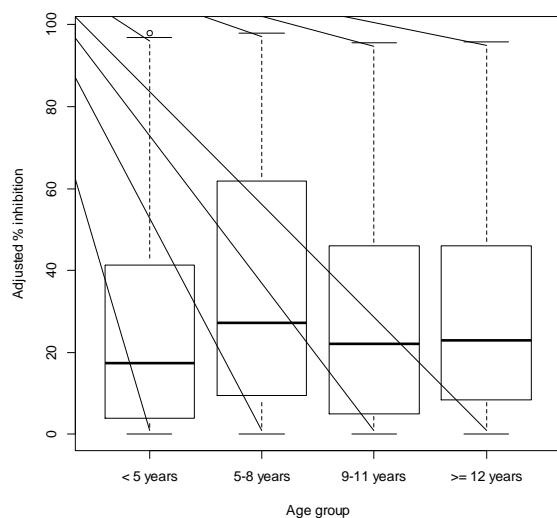
The prevalence of different antibody concentrations is depicted in figure 3.



**Figure 3.** Frequency histogram of CRCoV titres in 999 New Zealand dogs.

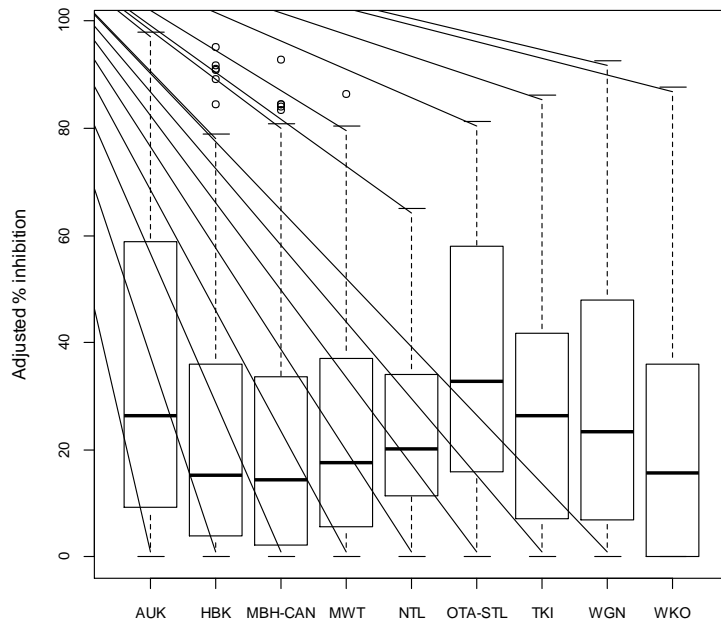
As can be seen, the majority of dogs have very low or "negative" antibody concentrations. Though we have yet to define what assay value represents a true positive, it is likely to be values above 30%.

Unlike in studies in the UK, antibody concentrations did not vary according to age (Figure 4.)



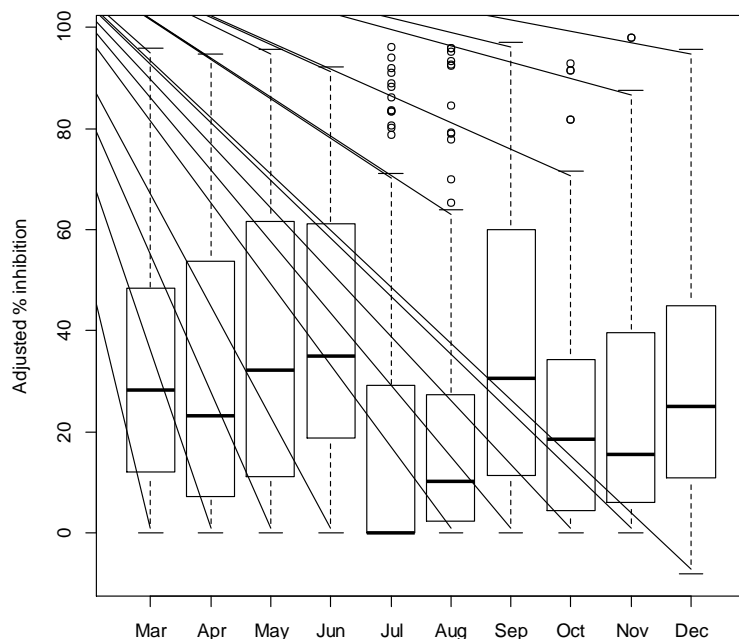
**Figure 4.** CRCoV titres in 999 New Zealand dogs according to age category

To test for differences in risk of exposure to CRCoV in NZ, we tested the significance of different titres according to region (Figure 5). Titres in dogs from Auckland tended to be higher than dogs in Marlborough-Canterbury, and dogs in the Manawatu regions. However, although there were some differences between regions, the differences are unlikely to be enough to represent real differences in risk of infection.



**Figure 5.** CRCoV titres in 999 New Zealand dogs according to region. Titres in dogs from the Auckland region were significantly higher than those in Marlborough-Canterbury (MBH-CAN), and dogs in the Manawatu (MWT) regions ( $P < 0.05$ ).

Previous studies in the US and UK have suggested a significant seasonal trend to the spread of CRCoV, whereby infection is greatest during the winter months. In NZ, it appears that the months with the *lowest* titres are July and August, whereas the other months do not appear to vary in a consistent pattern (Figure 6).

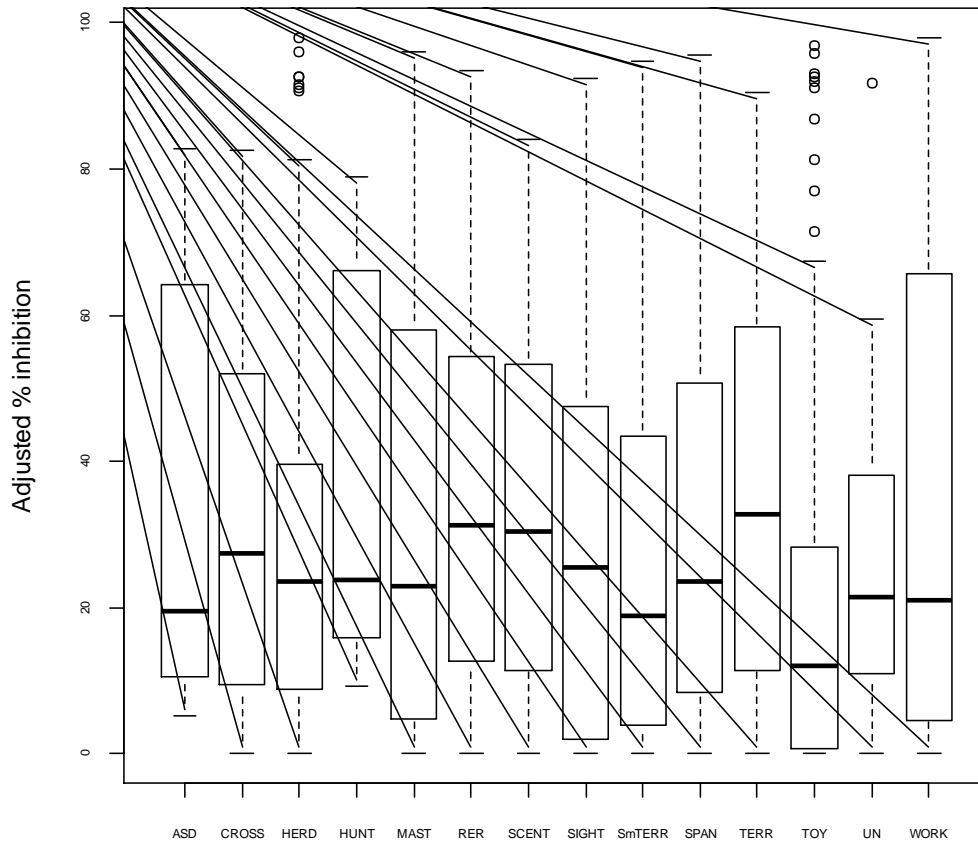


**Figure 6.** CReCoV titres in 999 New Zealand dogs according to month of sampling. The differences between months was significant ( $P < 0.05$ ), with July and August being significantly lower than all other months except October and November.

There was no significant difference in CReCoV titres between sexes. Predictably, there were many different breeds represented in the 999 random samples. In order to detect breed differences, dogs were categorized into 15 breed groups, based on published genetic similarities. The groupings and numbers of dogs are presented in the table below:

Breed_group	Code	N
<b>Ancient and spitz dogs</b>	ASD	15
<b>Cross breed</b>	CROSS	51
<b>Herding dog</b>	HERD	108
<b>Hunting dog</b>	HUNT	15
<b>Mastiff-like dog</b>	MAST	144
<b>Retriever</b>	RER	152
<b>Scent hound</b>	SCENT	22
<b>Sight hound</b>	SIGHT	46
<b>Small terrier</b>	SmTERR	119
<b>Spaniel</b>	SPAN	57
<b>Terrier</b>	TERR	33
<b>Toy dogs</b>	TOY	153
<b>Unknown</b>	UN	22
<b>Working dog</b>	WORK	78

Greyhounds were included in the "Sight hound" category. When dogs were grouped according to breed, the only significant difference between breed categories was that Toy breeds had a lower titre than retrievers (Figure 7,  $P < 0.05$ ).



**Figure 7.** CRCoV titres in 999 New Zealand dogs according to breed. Using a multiple comparison test after a Kruskal-Wallis test, toy breeds (TOY) had a lower titre than retrievers (RER)  $P < 0.05$ , whereas all other comparisons were not significantly different.

### Virus isolation

Multiple attempts to isolate live virus from clinical samples have been made to date. So far we have not yet successfully grown any respiratory virus in the laboratory. We have 4 samples that have produced very weak PCR results for CRCoV, when the samples were grown on specialized cells, that are ideal for isolating coronaviruses (HRT-18 cells). We are continuing to attempt viral isolation.

## Discussion

Since our preliminary report in 2011, we have obtained several more samples from affected dogs, but significantly fewer than hoped, especially from Racing Greyhounds. None-the-less, we have continued to affirm previous suspicions of the significance of some pathogens, and perhaps as importantly, of the lack of significance of others.

Techniques to detect multiple organisms are needed when investigating ACIT because of the multiple potential pathogens, and the inability to distinguish between the different organisms based on the clinical signs. We utilised a commercial laboratory (IDEXX) with an established, and ostensibly validated quantitative PCR assay that is capable of detecting 10 different pathogens. It was our hope that the assay would give us the best diagnostic value for the dollar. A negative result from a clinical sample can be due to poor sensitivity of the test, poor sample handling, lack of shedding of the causative pathogen, and the presence of a pathogen that is not included in the test panel. It is not possible for us to know which explanation is more common for the samples we have tested, but the discrepancy between our own in-house CAV-2 PCR, and the IDEXX qPCR suggests that assay sensitivity is at least partly to blame.

### *Mycoplasma cynos*

*Mycoplasma spp.* can be isolated from approximately a third of cases of lower respiratory tract disease, and have been incriminated in sporadic cases of serious pulmonary disease.<sup>2,3</sup> In the United Kingdom, dogs entering boarding kennels frequently experience an increase in serum antibodies to *M.cynos* soon after admission, and the majority of those experience signs of respiratory disease.<sup>4</sup> Equally, asymptomatic carriage is common, and mycoplasmas are thought to be part of the normal bacterial flora in the upper respiratory tract, but there are conflicting reports about the presence of mycoplasmas in the lower respiratory tract of healthy dogs.<sup>5</sup> In one study the lungs of up to 27% of healthy dogs were colonized, whereas in another, no mycoplasmas were detected in the lower respiratory tract of healthy dogs.<sup>5,6</sup>

Culture of *Mycoplasma sp.* is difficult, and at present, we have utilised PCR for detection, which does not guarantee infection, only the present of *M.cynos*-specific DNA. At present then, we cannot state that infection with *M. cynos* caused kennel cough in those dogs. It is equally possible that, coming from the same kennel, they were all colonised by the organism but it was not responsible for their disease. Based on the low rate of detection (9.7%, with a single cluster of infections), uncertainty as to the significance of it as a primary pathogen, and the difficulties working with the organism in the laboratory, we have not pursued this organism as an important cause of ACIT in NZ.

### *Bordetella bronchiseptica*

*B. bronchiseptica* was detected in only 3.8% of those samples tested. Unlike for some of the viruses, clinically affected dogs if sampled in the presence of clinical signs, *should* shed sufficient bacteria to be easily identified. Therefore, at this stage we do not believe that *B.bronchiseptica* is an important pathogen in these dogs. This is an important

conclusion, because most of the "Kennel Cough" vaccines contain *B. brochiseptica* as the sole organisms (some also contain parainfluenza virus). When referred to as the "kennel cough vaccine", it suggests protection against the organisms that cause kennel cough, when clearly that is not true. It may be that widespread vaccination, especially amongst racing greyhounds, has reduced the prevalence of *B. brochiseptica* to insignificant levels. Alternatively, it may be that the bacteria has simply been uncommon during the testing period, or even that it has never been an important pathogen in NZ. Whatever the explanation, it is clear that owners need to be educated as the narrow protection that the *B. brochiseptica* vaccine provides, and that other organisms are more likely to be more significant.

#### *Canine parainfluenza virus*

DNA from CPIV-3 was detected in 3 dogs, one of which was also the *B. bronchiseptica* positive dog. Shedding of this virus is possible following vaccination with either the injectable DHPPi, or intranasal combined CPIV-3/*Bordetella* vaccine. The finding of DNA from both organisms suggests possible vaccination, although the owner of the dog claimed the dog had not been recently vaccinated.

#### *Canine herpes virus-1*

One dog positive for canine herpes virus-1, and this is the first evidence of a possible association between CHV and kennel cough in New Zealand since the 1970's.

#### *Canine influenza virus*

So far, we have not detected DNA from CIV in clinically affected greyhounds. This would be consistent with the afore mentioned serosurvey, which did not detect antibodies to CIV in any of the 251 serum samples.<sup>1</sup>

#### *Streptococcus zooepidemicus*

We have neither detected DNA, nor isolated *Strep. Zooepidemicus* from affected greyhounds. Previous work by Els Acke has determined that the bacteria can be isolated from dogs in NZ, and it has been associated with acute, and sometimes severe tracheobronchitis and pneumonia. Our conclusion is that the bacteria is not a common or significant cause of disease in NZ dogs.

#### *Canine distemper, and Adenovirus 2*

Widespread vaccination has likely made these two viruses rare, and both may have been eliminated from NZ. That is not the case in other countries, and there is potential for both viruses to be reintroduced into NZ through infected, asymptomatic dogs. The large number of CAV-2 positive results in our in-house PCR is almost certainly the result of detection of vaccine virus.

#### *Canine respiratory coronavirus*

A serosurvey of 251 pet dogs in New Zealand in 2009, based on a convenience sample of serum submitted to a diagnostic laboratory (New Zealand Veterinary Pathology Ltd), reported a seropositive rate to CRCoV of 29%.<sup>1</sup> In our larger, randomised and year long

survey, we have found a very similar prevalence. In addition, sampling affected greyhounds in the acute phase, and then 3-4 weeks later reveals significant increases in antibody concentrations, which is strongly supportive of CRCoV as a cause of disease in a significant number of the dogs. We have not yet detected CRCoV DNA in clinically affected greyhounds. Nor have we convincingly managed to isolate the virus in cell culture. It is important that we do isolate and genetically sequence the virus found in New Zealand, so that we can predict if vaccines currently under development might be effective in this country. We have evidence that canine parvovirus has developed differently in this country compared with the rest of the world, and might be true for other canine viruses.<sup>7</sup> Though uncertain, it would be foolish for us to assume protection by vaccines manufactured from isolates in other countries.

Contrary to reports in other countries, there was no difference in antibody titres between dogs of different ages. In addition, fewer dogs were seropositive during the midwinter months than at other times of the year, which is opposite to the UK, where the winter months are the period of greatest exposure. This could well be due to a difference in kennelling during holidays, where in the UK it might be less common to kennel dogs during the summer than in NZ where summer coincides with the Christmas break. Alternatively, the differences in age and seasonal patterns could indicate other risk factors unique to NZ. However, the lower risk in toy-breeds would be consistent with the lower rate of kennelling with those breeds. We have insufficient data specific to Racing Greyhounds to determine if the patterns of disease in those dogs is different from the general dog population, but none of the results from our studies so far suggest that they differ for ACIT.

#### *Student support*

As a result of the research funding provided by the NZGRA, one Master's student has completed her research and is submitting her thesis, and a PhD student is well into her thesis work.

#### **Future and on-going research**

We have two key on-going research objectives that build upon the work described here:

1. Isolate and genetically sequence the CRCoV that is present in NZ dogs
2. Submit as many samples from healthy and diseased dogs as we can, to a more comprehensive "multi-pathogen" detection assay.

To achieve the first aim, we will continue our existing cell culture detect methods, coupled with PCR to confirm the presence of the virus, and will continue to recruit swabs from clinically affected dogs.

We are still in discussion with the New Zealand Genomics Service at the University of Otago as to the best method to detect known, and unknown respiratory pathogens in the samples we have obtained. There are several new, exciting, yet expensive techniques available, and given the novelty of these techniques, we will proceed with caution.



## **Recommendations**

It is highly likely that many, perhaps most of the outbreaks of ACIT in racing greyhounds at or following race meetings are not due to *Bordetella bronchiseptica* or Parainfluenza virus. Thus many dogs will not be immunised against the causative organism. This means that cleaning and disinfection of the kennel environment is essential. It should be recognised that the effectiveness of any disinfection procedure is dependent on initial cleaning to remove contaminated materials. The respiratory coronaviruses, for instance, remain infectious in faeces for 2 days. However, CRCoV, like most of the respiratory viruses, is easily killed by common disinfectants after a 1 minute contact time in ideal settings, although contact times of at least 30 minutes are recommended in field settings such as kennels. Suitable disinfectants include:

1. Sodium hypochlorite at a concentration of 1,000 parts per million. Using a 3% sodium hypochlorite solution, that equates to adding 35mL of bleach to 1 litre water. This can be increased to 175mL per litre of water if desired.
2. Potassium peroxymonosulfate (e.g. Trifectant R or Virkon-SR).

## **Conclusions**

Despite the considerable efforts of the team at Massey, and continued collection of samples by Greyhound veterinarians and other veterinarians around the country, we are unable to confidently identify the key causative organisms of ACIT in NZ racing greyhounds. That this uncertainty persists despite the financial commitment made by the NZGRA is not lost on the researchers, yet it remains a characteristic of much of scientific endeavours. None-the-less, we are gaining in confidence that the canine respiratory corona virus is a common and important pathogen, and that the organisms included in the vaccines typically referred to as the "kennel cough vaccines" do not appear to be key players. This point should be emphasised to the industry. In the absence of certainty, we recommend the persistence of current measures for the quarantine of dogs, for the stand-down periods, but recommend that kennels at the track be thoroughly disinfected following an outbreak. We hope in the near future to isolate and describe the CRCoV present in NZ, and it may be that the introduction of an effective vaccine will have a significant impact on racing dog health in the future.

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