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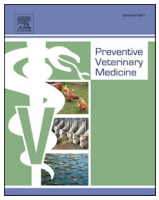

# Seroprevalence of canid alphaherpesvirus-1 and associated risk factors in domestic dogs in North Queensland, Australia

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ABSTRACT

Canid alphaherpesvirus-1 (CaHV-1) may cause a highly fatal haemorrhagic disease in neonatal pups and is associated with reproductive, respiratory and ocular disease in older dogs. Although assumed to have a world-wide distribution, there have been few reports of CaHV-1 in Australia. The aim of this study was to investigate the seroprevalence of CaHV-1 in household dogs in a residential suburb in Townsville, as well as in dogs attending two dog shows in the region. Study participants were recruited through door-to-door non-probability sampling (Douglas dogs, n = 185) or invited to participate (Show dogs; n = 76). Dog owners completed a questionnaire that investigated possible risk factors for recent exposure to CaHV-1. A serum sample from each dog was assayed for anti-CaHV-1 antibodies using a commercially available ELISA. Associations between sero-positive dogs and owner-reported risk factors were analysed using univariable analysis and multivariable logistic regression models. The seroprevalence of CaHV-1 was 11.4 % (95 % CI 6.8–15.9 %) and 17.1 % (95 % CI 5.5–28.8) for the Douglas and Show dogs, respectively, with a pooled seroprevalence of 13 % (95 % CI 8.3–17.7 %). Dogs that had suffered from conjunctivitis within the previous 3 months or were involved in breeding were more likely to be seropositive to CaHV-1. No other significant risk factors were identified. In conclusion, CaHV-1 is circulating in dogs in North Queensland and may be contributing to foetal and neonatal losses in this region.

## 1. Introduction

Over 50 years ago, Carmichael et al. (1965a) described a viral disease that caused severe illness and death in young pups. Newly born pups-inoculated with the virus (n = 12; inoculated 24–36 hours after birth) showed similar duration and severity of disease, regardless of the size of the initial viral inoculum, and died 6–9 days post inoculation following a brief period of clinical illness that included anorexia and incessant crying. Widespread focal necrosis and haemorrhage were observed on postmortem. In contrast, pups infected after 2 weeks of age were reported to show, at most, mild clinical signs following an incubation period of around one week. The virus was soon identified as a herpesvirus (Carmichael et al., 1965b; Spertzel et al., 1965; Stewart et al., 1965) and is now recognised as canid alphaherpesvirus-1 (CaHV-1) (Lewin et al., 2020).

In adult dogs, canid alphaherpesvirus-1 (CaHV-1) is linked to respiratory (Karpas et al., 1968) and ocular disease (Ledbetter et al., 2009a; Ledbetter, 2013), and reproductive disorders including vesicular mucosal lesions on the external genitalia (Hill and Mare, 1974; Hashimoto et al., 1983), infertility (Poulet et al., 2001), stillbirth and abortion (Poste and King, 1971). Diagnosis of CaHV-1 is based on typical post mortem findings in neonates, including widespread petechiae and viral inclusion bodies on histopathology, as well as virus isolation, PCR and serology (Lewin et al., 2020). As is typical for a herpesvirus, CaHV-1 establishes latency after the initial infection, with viral DNA detected in a number of tissues including the trigeminal and lumbosacral ganglia, tonsils and parotid salivary gland (Burr et al., 1996). Reactivation of the virus can be induced by corticosteroid medication (Okuda et al., 1993a, 1993b).

The seroprevalence of CaHV-1 has been reported in a number of countries, including the UK (Reading and Field, 1998), Belgium (Ronsse et al., 2002), The Netherlands (Rijsewijk et al., 1999), South Africa (Nöthling et al., 2008) and Italy (Pratelli et al., 2014). Reports of suspected (Huxtable and Farrow, 1970) or confirmed (Geldard et al., 1971;

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Watt et al., 1974; Reubel et al., 2002) CaHV-1 in Australia have been scant, and no studies on the prevalence of the virus in this country have been published previously.

The aim of the current study was to investigate the seroprevalence of CaHV-1 in dogs in Northern Queensland, Australia. In addition, a questionnaire was designed to examine possible risk factors associated with dogs found to be seropositive for CaHV-1.

## 2. Materials and methods

### 2.1. Study design

This study was approved by the [information removed] Animal and Human Ethics Committees (approval numbers A2797 and H8626, respectively). The study comprised two groups of dogs: one group consisted of dogs living in the suburb of Douglas (Douglas dogs) and a second group consisted of dogs sampled at dog shows (Show dogs) in northern Queensland. At the time of this study, no vaccines against CaHV-1 had been approved for use in Australia.

#### 2.1.1. Douglas dogs

The suburb Douglas was chosen as a representative sample of the Townsville population, due to the varying socioeconomic status of residents in the suburb. The suburb is situated south-west of the city centre and housed a population of 7780 in 2021 [25]. Study participants were recruited through door-to-door non-probability convenience sampling between August 2022 and November 2022.

Communication regarding this research was conveyed to the Douglas community via a community social media page, prior to and during the sampling period. In addition, a one-page flyer was delivered to residents' mailboxes approximately one week prior to the research team arriving in that area of the suburb. Each household was approached requesting study participation if they owned a dog over the age of one year. Only one dog per household was permitted to avoid clustering in the study design. If there was no response to a door knock or if the resident declined study participation, the research team moved on to the next home on the street. Most surveying took place between 3 pm and 7 pm in order to increase the likelihood of residents being at home at that time.

#### 2.1.2. Show dogs

Dogs were recruited from two dog shows, held at Woodstock, northern Queensland in August 2022 and at Proserpine, in the Whitsunday region of Queensland, in October 2022. Before each show, communication regarding this research was disseminated to show entrants by the responsible Kennel Clubs, which was endorsed by the parent organisation, Dogs Queensland. Show participants were invited to volunteer any dogs over the age of one year. More than one show dog per owner could be submitted for sampling.

### 2.2. Sample collection and storage

Up to 5 ml of blood was collected from the cephalic vein of each dog, using a 22-gauge needle. The blood was immediately transferred to a plain (serum) vacutainer tube and allowed to clot at 4 °C overnight, followed by centrifugation and aliquoting of serum into cryovials for storage at −80 °C.

### 2.3. Questionnaire survey

A structured questionnaire was designed in Epi Info™ version 7.2.5.0 and completed by dog owners at the time of sample collection (Appendix S1). The questionnaire comprised 20 questions to establish the dog's demographic details (sex, neuter status, breed and age), current residence and management (including the number of dogs normally present on the property), reproductive history, any relevant clinical signs observed over the past 12 months, and the dog's social habits (for example, how often the dog was walked or socialised with other dogs).

### 2.4. Detection of serum antibodies

Serum samples were tested for IgG antibodies to CaHV-1 using a commercially available ELISA, according to the manufacturer's instructions (BV European Veterinary Laboratory, D1004-AB01). Samples were diluted 1:150 in ELISA buffer prior to testing. Absorbance values were measured at 450 nm using 620 nm as reference on a SPECTROstar Nano plate reader (BMG Labtech). A sample-to-positive control ratio of $\geq$0.23 was considered positive, as per the manufacturer's recommendation.

### 2.5. Data analysis

The data was analysed as two independent populations (Douglas dogs and Show dogs) and as a pooled population. Apparent prevalence was calculated as the number of CaHV-1 seropositive dogs divided by the population at risk. For the Douglas dogs, the 95 % confidence interval for true prevalence was calculated assuming a sensitivity and specificity of 100 % according to Thrusfield (2005) using Eqs. (1) and (2):

Eq. (1) to calculate the Standard Error (SE) of the apparent prevalence:

$$SE(\hat{p}) = \sqrt{\frac{\hat{p}(1-\hat{p})}{n}} \quad (1)$$

Where $\hat{p}$ is the apparent prevalence and n is the sample size.

Eq. (2) to calculate the 95 % confidence interval for the true prevalence of disease:

$$95\%Confidence\ Interval(\hat{p}) = p \pm 1.96 \times SE(\hat{p}) \quad (2)$$

Where SE is the standard error calculated in Eqs. (1) and 1.96 is the Z value for a 95 % confidence interval.

For the Show dogs and Pooled dogs, where more than one dog per owner was bled, the 95 % confidence interval for the seroprevalence was calculated taking into account clustering within the data using Eqs. (3) and (4) (Thrusfield (2005)

$$\hat{P} - 1.96 \left\{\frac{C}{T}\sqrt{\frac{V}{C(C-1)}}\right\}, \quad \hat{P} + 1.96 \left\{\frac{C}{T}\sqrt{\frac{V}{C(C-1)}}\right\} \quad (3)$$

Where:
C = number of clusters in the sample
T = total number of animals in the sample
and:

$$V = \hat{P}^2(\Sigma n^2) - 2\hat{P}(\Sigma nm) + (\Sigma m^2) \quad (4)$$

Where:
n = number of animals sampled in each cluster
m = number of diseased animals sampled in each cluster

As the sensitivity and specificity of the ELISA test kits used could not be given by the manufacturers, the true prevalence was also modelled as a Beta distribution function (Eq. (5)) using the add-in software program @RISK 8.1 in MS Excel (Palisade company LLC). The programme was set up for 10,000 iterations using the Latin Hypercube sampling technique (Vose, 2008). The Beta distribution estimates the probability of the occurrence of a specific event from an observed number of events/successes (s) in a specific number of trials (n) and can be used to describe the uncertainty or random variation of a probability, fraction or prevalence (Vose, 2008).

Beta distribution for prevalence : (5)

Beta (α,β) Where α = s + 1 and β = n-s+1 and s is the observed

number of successes (s) (number of CaHV-1 positive) in a specific number of trials (n) (Sample Size).

Questionnaire data were entered into the database program Epi Info™ version 7.2.5.0. Analysis of data was carried out using Epi Info™, Microsoft Excel (version 2302) and the statistical software program NCSS (Hintze, J., 2023, Kaysville, UT, USA). The associations between seropositive dogs and various risk factors were analysed using Odds ratios and Chi-square tests (i.e. univariable analysis). Risk factors included in the questionnaire that were used for the univariable analysis are listed in Table 1.

Risk factors with a P-value $< 0.20$ on univariable analysis were selected to be included in the multivariable logistic regression models (Katz, 1999). A hierarchical stepwise forward elimination process with switching of variables based on log likelihood values was used to determine the best fitting logistic regression model. NCSS has a built-in algorithm that does the selection process and several iterations were run before the final model was selected, which contained the best fitting log likelihood value and only the variables with a P-value $< 0.05$ on the Wald test (Armitage and Berry, 1994). Collinearity between risk factors was also screened for and it was decided to model dogs with a risk factor present in the last 3 month and dogs with the same risk factor present in the last 12 months as separate models.

## 3. Results

In total, 261 dogs were sampled for this study.

### 3.1. Dog demographics

#### 3.1.1. Douglas dogs

The study population included a total of 185 dogs resident in the suburb of Douglas. It is estimated that more than 90 % of Douglas homes were approached during this study. The average number of dogs on each property surveyed was 1.5 dogs (range 1–4 dogs). Surveyed dogs had a mean age of 6 years (SD = 3.8, SE = 0.28; range 1–16.5 years), and comprised 9 % entire females, 42 % spayed females, 9 % entire males and 40 % neutered males. The origin of most dogs (30 %) was unknown or not disclosed, with 27 % coming from a council registered breeder, 22 % from a national kennel club-registered breeder, 15 % from a welfare agency and 6 % from an animal shelter. Forty-two breeds were reported, with the majority of dogs being a mixed breed (40 %). The most frequent breed of dogs was the Border Collie (6.5 %).

#### 3.1.2. Show dogs

Seventy-six dogs were tested at two dog shows in northern Queensland. The average number of dogs on dog owners' properties was 7 dogs (range 1–20 dogs). The group had a mean age of 4 years (SD = 3, SE = 0.36; range 1–15.7 years), and comprised 47 % entire females, 13 % spayed females, 35 % entire males and 4 % neutered males. Thirty-seven owners were surveyed of which 9 had one or more dogs positive (24 %). The average and median number of dogs per owner was 2 with a mode of only 1 dog per owner. For owners that had one or more positive dogs, the most frequent proportion of positive dogs per owner was 50 % with a range from 33 % to 100 %. The origin of most dogs (96 %) was a national kennel club-registered breeder with 1 % coming from an animal shelter. The remaining 3 % were from other sources. Thirty-three breeds were reported, with only 1 % being a mixed breed. The most frequent breed of dogs was the Italian Greyhound (8 %) followed by the Border Collie (7 %) and Dobermann (7 %).

More detail on the breeds identified and the proportion of these within each survey population can be found in the supplementary material (Appendix S1).

**Table 1**
Risk factors included in the univariable analysis.

| Risk Factor* | Risk Factor* |
|---|---|
| Abortion | Genital lesions<3 m |
| Abortion<3 m | Infertility |
| Any Breeding | Infertility<3 m |
| Conjunctivitis | Nasal discharge |
| Conjunctivitis<3 m | Nasal discharge<3 m |
| Corticosteroid meds | New animals introduced |
| Coughing | Origin of animal |
| Coughing<3 m | Planned breeding |
| Discharge from eyes | Pregnancy loss |
| Discharge from eyes<3 m | Pregnancy loss<3 m |
| Dog show or sports | Sex and neuter status |
| Dog training | Sneezing |
| Eye ulcer | Sneezing<3 m |
| Eye ulcer<3 m | Unplanned breeding |
| Genital lesions | |

* Risk factors relate to the previous 12 months unless indicated to have occurred in the previous 3 months (<3 m).

### 3.2. Seroprevalence of CaHV-1

For the Douglas dogs, the seroprevalence of CaHV-1 was 11.4 % (21/185) with a 95 % confidence interval for the true prevalence ranging from 6.8 % to 15.9 %. For the Show dogs, the seroprevalence of CaHV-1 was 17.1 % (13/76) with a 95 % confidence interval for the true prevalence ranging from 5.5 % to 28.8 %. The Pearson's Chi-Square was used to establish if there was a statistical difference between the seroprevalence of Douglas and Show dogs. No difference could be shown between the prevalence of CaHV-1 in both groups at $\alpha = 0.05$ (2-Sided) (Chi-square = 1.57; df = 1; p = 0.21).

Combining the two study groups, the apparent seroprevalence of CaHV-1 was 34/261 = 13 % with a 95 % confidence interval for the true prevalence of 8.3–17.7 %.

The β distribution functions for the prevalence CaHV-1 in both groups, and when pooled, are shown in Fig. 1. These show the probability of the seroprevalence of canid alphaerpesvirus-1 (Y-Axis) in the sample population being less than a given seroprevalence (X axis) and account for the uncertainty and variability within the data.

#### 3.2.1. Douglas dogs

Univariable analysis of Douglas dog risk factors found dogs that had conjunctivitis in the past 3 months to be associated with CaHV-1 seropositive status (OR = 8.9, 95 %CI:1.7–47.6; $\chi^2 = 9.2$, P<0.01) at $\alpha = 0.05$ for the $\chi^2$ test. Evidence of discharge from eyes (OR = 3.2, 95 % CI:0.8–13.5; $\chi^2 = 2.9$, P = 0.09) was found to be associated with CaHV-1 seropositive dogs at $\alpha = 0.10$.

The logistic regression model for Douglas dogs (Table 2) (estimated logistic regression model for CaHV-1 ELISA Positive = Yes: −0.14 + 2.15*(Conjunctivitis in last 3 m = "Yes")) also showed that there was an association between dogs positive for CaHV-1 and dogs that had a reported conjunctivitis within the past 3 months (OR = 8.6, Wald P = 0.01). The final Model had an $R^2 = 0.04$ and log-likelihood = −62, bearing in mind that in logistic regression the relationship between X and Y may be nonlinear and if the dependent variable has more than two unique values, there are several regression equations (NCSS, 2023). Despite the small $R^2$ value the model correctly classified the outcome 88 % of the time (Table 3). None of the other risk factors, nor age, sex and neuter status, showed an association.

Risk factors in the questionnaire pertaining to Douglas dogs that were not included in the analysis because of insufficient data were: pregnancy loss, infertility, abortion, genital lesions, nasal discharge, eye ulcer, and unplanned breeding.

#### 3.2.2. Show dogs

Univariable analysis of Show dog risk factors could find no significant association of any of the risk factors at $\alpha = 0.05$. However, planned breeding (OR = 3.2, 95 %CI: 0.89–11.5; $\chi^2 = 3.4$, P = 0.07) and evidence of coughing in the past 3 months (OR = 5.6, 95 %CI: 0.71–43.6; $\chi^2$

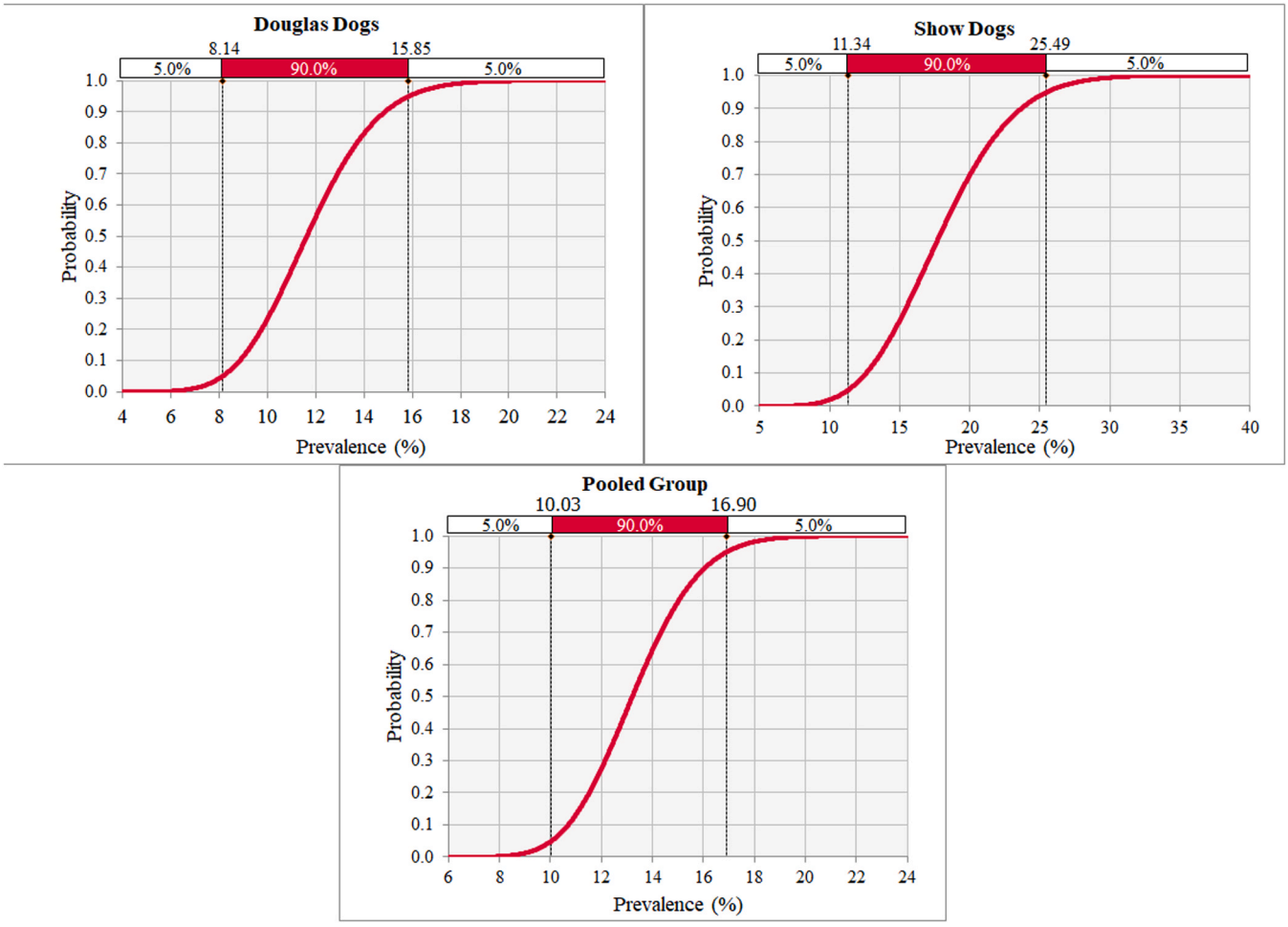


**Fig. 1.** The probability of the seroprevalence of canid alphaerpesvirus-1 in the sample population (y-axis) being less than a given seroprevalence (x-axis) for each sampled group.

**Table 2**
Logistic regression results for risk factors associated with CaHV-1 seropositive Douglas dogs.

**Coefficient Significance Test**

| Independent Variable | Regression Coefficient b(i) | Standard Error Sb(i) | Wald Z-Value H0: β = 0 | Wald Prob Level | Odds Ratio Exp(b(i)) |
|---|---|---|---|---|---|
| B0: Intercept | −0.13 | 0.09 | −1.50 | 0.13 | 0.87 |
| B1: (Conjunctivitis in last 3 m = "Yes") | 2.15 | 0.85 | 2.52 | 0.01 | 8.6 |

**Odds Ratios Report**

| Independent Variable | Regression Coefficient b(i) | Odds Ratio Exp(b(i)) | Lower 95 % Confidence Limit | Upper 95 % Confidence Limit |
|---|---|---|---|---|
| B0: Intercept | −0.14 | 0.87 | 0.73 | 1.04 |
| B1: (Conjunctivitis in last 3 m = "Yes") | 2.15 | 8.56 | 1.60 | 45.59 |

= 3.2, P = 0.07) were significant on the $\chi^2$-test at α = 0.10.

For Show dogs, none of the logistic regression models found any of the risk factors included in the models to be significantly associated with the presence of CaHV-1 antibodies. Dogs that had been purposively bred (planned breeding) showed some evidence of an association (OR = 3.2, Wald P = 0.7) at a 10 % level of significance.

**Table 3**
Response analysis detailing the accuracy of the regression model for Douglas dogs.

| Categories | Count | Unique Rows | Prior | Act vs Pred $R^2$ | % Correctly Classified |
|---|---|---|---|---|---|
| No | 157 | 8 | 0.5 | 0.05 | 98 |
| Yes | 21 | 7 | 0.5 | 0.05 | 14 |
| Total | 178 | 15 | | | 88 |

Risk factors in the questionnaire pertaining to show dogs that were not included in the analysis because of insufficient data were: pregnancy loss, infertility, abortion, genital lesions, nasal discharge, eye ulcer, unplanned breeding, discharge from eyes, sneezing, corticosteroid medication, and conjunctivitis.

### *3.2.3. All dogs (Pooled)*

Univariable analysis of risk factors when the groups were pooled found conjunctivitis in the past 3 months (OR = 5.4, 95 %CI: 1.2–25.6; $\chi^2$ = 5.7, P = 0.02), planned breeding (OR = 2.5, 95 %CI: 1.1–5.8; $\chi^2$ = 5.1, P = 0.02) and any breeding (Has your dog ever been involved in breeding?) (OR = 2.2, 95 %CI: 0.97–2.8; $\chi^2$ = 3.7, P = 0.05) to be significantly associated with CaHV-1 seropositive dogs at α = 0.05. Conjunctivitis in the past 12 months (OR = 2.9, 95 %CI: 0.85–9.8; $\chi^2$ = 3.2, P = 0.08) was significant at α = 0.10.

Since conjunctivitis in the past 3 months (Model 1, Table 4) and conjunctivitis in the past 12 months (Model 2,Table 6) show collinearity, these were placed into two separate models. Both models showed the % correctly classified by the model to be ≥75 % (Table 5 and Table 7). Both models showed breeding (Has your dog ever been involved in breeding?) and a history of conjunctivitis in the last 3 or 12 months to be significantly associated with dogs with CaHV-1 antibodies. When analysed further, breeding was divided into artificial insemination and natural breeding. Natural breeding failed to show any association on the univariable analysis and was not included in the logistic regression. Artificial insemination was then modelled in place of "any breeding" and "planned breeding" and although it came up strongly in the regression model as the second most significant variable (Wald Z-value = 1.31; Wald P-value = 0.19; OR = 2.10), it dropped out in the final analysis leaving "conjunctivitis in the last 3 months" as the only variable associated with CaHV-1 in this model.

Risk factors in the questionnaire that were not included in the analysis because of insufficient data were: pregnancy loss, infertility, abortion, genital lesions, nasal discharge, and unplanned breeding.

## 4. Discussion

The current study demonstrates that CaHV-1 is circulating in North Queensland dogs. The seroprevalence of CaHV-1 in this study is modelled at a 95 % probability of being less than 17 % (median = 13 % and mean = 8 %) for both groups combined, which is lower than the 22 % reported in breeding dogs in South Africa (Nöthling et al., 2008), 39.3 % reported in The Netherlands (Rijsewijk et al., 1999), 45.8 % reported in Belgium (Ronsse et al., 2002), and 94 % reported in England (Reading and Field, 1998), but similar to the 14.6 % seroprevalence of CaHV-1 in samples submitted to a veterinary laboratory in southern Italy (Pratelli et al., 2014). A second Italian study, based on breeding dogs in the northwest, reported a seroprevalence to CaHV-1 of 50.3 % (Rota et al., 2020). Variation in CaHV-1 seroprevalence over geographical areas has also been reported within a single study, with the proportion of positive samples from four different veterinary clinics in Norway ranging from 58.5 % to 98 % (Krogenæs et al., 2012). Research on how the seroprevalence of CaHV-1 may fluctuate within a single district over time is currently lacking, although some evidence suggests a seasonal influence (Krogenæs et al., 2012, 2014). Additional reasons for the wide range in seroprevalence reported may include differences in assay sensitivities, threshold values and sampling frames.

Undertaking a survey of suburban dogs has eliminated some of the bias that is associated with industry-based surveys (e.g. a breeding kennel, animal shelter, veterinary clinic or diagnostic laboratory). Sampling at a veterinary clinic or laboratory could increase the proportion of dogs that are immunosuppressed due to chronic disease, and therefore more likely to be CaHV-1 seropositive (Ronsse et al., 2002). Similarly, a higher CaHV-1 seroprevalence has been linked to breeding kennels, particularly those with at least six animals (Ronsse et al., 2004), and animal shelters (Yeşilbağ et al., 2012), although other studies found no link between CaHV-1 exposure and breeding status (Ronsse et al., 2002; Krogenæs et al., 2012) or kennel size (Nöthling et al., 2008). The seroprevalence of CaHV-1 in the Douglas dogs could not be shown to be statistically different (P<0.05) to that of Show dogs in the current study. This may be due to the relatively small number of Show dogs and resultant wide confidence intervals for true prevalence for this group. Krogenæs et al. (2014) found that dogs that attended dog shows were less likely to be seropositive to CaHV-1, perhaps due to heightened awareness of the risks of dog-to-dog contact by their owners. Other studies have found either no link between CaHV-1 titre status and show attendance (Krogenæs et al., 2012) or an increased risk of CaHV-1 exposure with show attendance (Gracin et al., 2023).

In the current study, conjunctivitis within the past 3 months showed the strongest link to positive CaHV-1 antibody status, among the variables studied. In addition, owner-reported ocular discharge within the past 12 months was associated with CaHV-1 seropositivity at the level of α = 0.10. Given the nature of the study, a cut point of α = 0.10 is probably still acceptable in showing that an association exists. In an early report, Poste and King (1971) described an outbreak of CaHV-1 in a kennel following the introduction of two puppies with "runny eyes". Male and female Beagles inoculated intrapreputially or intravaginally with CaHV-1 all developed genital lesions, and some developed a mild to severe conjunctivitis (Hill and Mare, 1974). Similarly, late pregnant bitches inoculated with CaHV-1 all developed conjunctivitis and a serous nasal discharge (Hashimoto et al., 1982). More recently, Ledbetter et al. (2009a) inoculated CaHV-1 topically onto a single eye per dog, which led to mild to moderate, bilateral conjunctivitis in all infected dogs; this clinical feature returned in some dogs following viral recrudescence induced by corticosteroid therapy (Ledbetter et al., 2009c). In addition, CaHV-1 was identified in conjunctival samples from 5 of 30 dogs (17 %) with naturally-occurring conjunctivitis, representing the most common viral cause of conjunctivitis in this study (Ledbetter et al., 2009b). The current study appears to be the first to link owner-reported occurrences of conjunctivitis with CaHV-1 antibody status.

In the current study, an association was found between CaHV-1 antibody status and dogs that had been involved in breeding when the data was pooled. The spread of CaHV-1 is thought to be primarily via oronasal contact, either directly or via aerosol, with venereal contact playing a secondary role (Ronsse et al., 2002; Krogenæs et al., 2014). Given that most breeding activities involve direct contact with other dogs, our results provide no evidence against this hypothesis.

In Australia, no vaccines against CaHV-1 are currently available. The relatively low seroprevalence of CaHV-1 detected in the current study may appear reassuring to breeders and veterinarians in Australia.

**Table 4**
Logistic regression results and Model 1 for risk factors associated with CaHV-1 Pooled seropositive dogs.

**Coefficient Significance Tests**

| Independent Variable | Regression Coefficient b(i) | Standard Error Sb(i) | Wald Z-Value H0: β = 0 | Wald Prob Level | Odds Ratio Exp(b(i)) |
|---|---|---|---|---|---|
| B0: Intercept | −0.25 | 0.13 | −2.02 | 0.043 | 0.78 |
| B1: (Planned = "Yes") | 0.86 | 0.43 | 2.01 | 0.044 | 2.37 |
| B2: (Conjunctivitis in past 3 m = "Yes") | 1.58 | 0.80 | 1.97 | 0.049 | 4.86 |

**Odds Ratios Report**

| Independent Variable | Regression Coefficient b(i) | Odds Ratio Exp(b(i)) | Lower 95 % Confidence Limit | Upper 95 % Confidence Limit |
|---|---|---|---|---|
| B0: Intercept | −0.25 | 0.76 | 0.61 | 0.99 |
| B1: (Breeding = "Yes") | 0.86 | 2.37 | 1.02 | 5.48 |
| B2: (Conjunctivitis in past 3 m = "Yes") | 1.58 | 4.86 | 1.01 | 23.42 |

**Estimated logistic regression Model 1 for CHV ELISA Positive = Yes**: 0.25 + 0.86*(Breeding = "Yes") + 1.58*(Conjunctivitis past 3 m = "Yes").
Model $R^2$ = 0.05; Model log-likelihood = −96.

**Table 5**
Response analysis detailing the accuracy of Model 1 for all dogs.

| Categories | Count | Unique Rows | Prior | Act vs Pred $R^2$ | % Correctly Classified |
|---|---|---|---|---|---|
| No | 220 | 5 | 0.5 | 0.04 | 84 |
| Yes | 34 | 5 | 0.5 | 0.04 | 35 |
| Total | 254 | 10 | | | 78 |

**Table 6**
Logistic regression results and Model 2 for risk factors associated with CaHV-1 Pooled seropositive dogs.

| **Coefficient Significance Tests** | | | | | |
|---|---|---|---|---|---|
| | **Regression** | **Standard** | **Wald** | **Wald** | **Odds** |
| Independent | Coefficient | Error | Z-Value | Prob | Ratio |
| Variable | b(i) | Sb(i) | H0: β = 0 | Level | Exp(b(i)) |
| B0: Intercept | −0.28 | 0.14 | −2.06 | 0.04 | 0.76 |
| B1: (Planned = "Yes") | 0.92 | 0.43 | 2.16 | 0.03 | 2.51 |
| B3: (Conjunctivitis = "Yes") | 1.08 | 0.63 | 1.71 | 0.09 | 2.95 |

| **Odds Ratios Report** | | | | |
|---|---|---|---|---|
| | **Regression** | **Odds** | **Lower 95 %** | **Upper 95 %** |
| Independent | Coefficient | Ratio | Confidence | Confidence |
| Variable | b(i) | Exp(b(i)) | Limit | Limit |
| B0: Intercept | −0.28 | 0.76 | 0.56 | 0.99 |
| B1: (Planned = "Yes") | 0.92 | 2.51 | 1.09 | 5.78 |
| B3: (Conjunctivitis = "Yes") | 1.08 | 2.95 | 0.85 | 10.16 |

**Estimated logistic regression Model 2 For Ca HV ELISA Positive = Yes**:-0.28 + 0.92*(Breeding = "Yes") + 1.08*(Conjunctivitis past 12 m = "Yes").
Model $R^2$ = 0.04; Model log-likelihood = −97.

**Table 7**
Response analysis detailing the accuracy of Model 2 for all dogs.

| Categories | Count | Unique Rows | Prior | Act vs Pred $R^2$ | % Correctly Classified |
|---|---|---|---|---|---|
| No | 220 | 5 | 0.5 | 0.03 | 81 |
| Yes | 34 | 5 | 0.5 | 0.03 | 38 |
| Total | 254 | 10 | | | 75 |

However, antibodies to CaHV-1, inoculated intra-ocularly, persisted for up to 8 months after primary infection but only 6 weeks after viral reactivation due to corticosteroid medication (Ledbetter et al., 2009c). Given the relatively short half-life of CaHV-1 antibodies, seroprevalence studies for this disease are likely an underestimate of the true disease burden (Rijsewijk et al., 1999; Verstegen et al., 2008; Ledbetter, 2013).

It has previously been suggested that there is a "6-week danger period" for CaHV-1, encompassing the final three weeks of pregnancy and the first three weeks of life (Evermann et al., 2011), although extending this to the entire gestation period may be prudent given that some evidence suggests an effect of the virus on fertility (Poulet et al., 2001). Primary or recurrent infection with CaHV-1 outside of the danger period causes subclinical or mild disease in most dogs, with more severe consequences generally limited to extremely old and/or immunocompromised individuals (Kawakami et al., 2010). In countries where CaHV-1 circulates efficiently, breeding animals may be more likely to develop immunity to the virus outside of the danger period (Krogenæs et al., 2012). In contrast, if the lower seroprevalence detected in the current study implies more sporadic viral spread and lower herd immunity in this region, then animals within the danger period for CaHV-1 may be less likely to have pre-existing immunity to the virus, increasing the need for strict biosecurity for pregnant, or potentially pregnant, bitches.

The current impact of CaHV-1 on fertility, abortion and neonatal death in Australian dogs is essentially unknown, with published reports limited to historical case studies (Huxtable and Farrow, 1970; Geldard et al., 1971; Watt et al., 1974). Sequencing of Australian isolates of CaHV-1 have demonstrated only minor sequence differences to isolates from other countries (Reubel et al., 2002; Lewin et al., 2020).

Limitations of this study include the limited sample numbers and geographical range. Further study of the seroprevalence of CaHV-1 in other areas of Australia is warranted and may vary substantially. The design of the current study also limited the results to prevalence data during a single period in time (spring/early summer), which provided no insight into the dynamics of CaHV-1 antibody levels in an individual dog over time, or within the population during different seasons. Notably, the ELISA assay employed in this study relied on a threshold for positivity, and data on the sensitivity and specificity of this assay are currently lacking. Clustering was accounted for in the calculation of prevalence but could not be fully addressed in the logistic regression and this may have introduced some slight bias in the Show dog and Pooled data analyses. However, the presence of one CaHV-1 seropositive dog in a household did not preclude seronegativity in co-housed dogs, therefore the impact of clustering on our results may have been minor. Finally, confounding and collinearity are common problems in these types of studies. In the current study, data was primarily dichotomous, which limited the use of stratification to account for confounding. However, the use of logistic regression minimised the effect of confounding in the final analysis. In addition, risk factors where collinearity was likely were separated in the final analysis to eliminate this as a source of bias.

In conclusion, CaHV-1 is circulating in domestic dogs in Queensland and is likely to be present in dog populations throughout Australia. In mature dogs, CaHV-1 should be considered in cases of conjunctivitis, where other causes have been excluded. Although the seroprevalence of CaHV-1 in Queensland is lower than that reported in some other countries, care should be taken to protect pregnant bitches, newborn puppies and immunocompromised dogs in Australia from CaHV-1 infection.

## Declaration of Competing Interest

The authors have no conflicts of interest to declare.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.prevetmed.2024.106304.

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