



Joyce, H., Oliver, J. A. C., Fricker, G., [Weir, W.](#) and Fleming, L. (2023) Keratouveitis in juvenile dogs and its presumed association with canine adenovirus infection. *Veterinary Ophthalmology*, 26(3), pp. 262-267. (doi: [10.1111/vop.13085](https://doi.org/10.1111/vop.13085))

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<https://doi.org/10.1111/vop.13085>

<https://eprints.gla.ac.uk/295760/>

Deposited on: 19 April 2023

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1 Title: Keratouveitis in juvenile dogs and its presumed association with canine adenovirus
2 infection

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20 Running title: Keratouveitis association with canine adenovirus

1 Abstract:

2 **Objective:** We hypothesized that keratouveitis still occurs despite current widespread use of
3 Canine adenovirus (CAV)-2 vaccinations and assessed the utility of CAV-1 and CAV-2 titers
4 in elucidation of its etiopathogenesis. **Animals Studied:** Nine dogs with unexplained
5 keratouveits (14 eyes) and nine control dogs. **Procedures:** The Animal Health Trust clinical
6 database was searched between 2008 and 2018 to identify cases of keratouveitis. Inclusion
7 criteria included known vaccination status, interval from vaccination to development of
8 clinical signs and availability of CAV titers. Cases were excluded if they were older than one
9 year of age, or other causative ocular pathology for corneal edema was identified. Nine age-
10 matched dogs without corneal edema but with CAV titers were included as controls. **Results:**
11 Mean CAV-1 and CAV-2 titers were not statistically different between dogs with
12 keratouveits and controls ($p=0.16$ and $p=0.76$, respectively). Three cases had CAV-1 titers
13 >5000 and two of these cases had rising convalescence titers (greater than an eleven-fold
14 increase) suggesting infection with wild-type CAV-1. The six other cases did not appear to be
15 associated with CAV infection or vaccination. **Conclusion:** Keratouveitis continues to occur
16 despite the advent of CAV-2 vaccinations. While this study found no evidence to indicate
17 CAV-2 vaccination causes keratouveitis, the data indicates that in a proportion of cases,
18 contemporaneous wild-type CAV-1 infection is a possible cause.

19 **Key words;** Canine Adenovirus, Keratouveitis, blue eye, CAV titers, Wild-type CAV-1
20 infection

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1 **Introduction:** Keratouveitis or the “blue eye” phenomenon in dogs, was first recognised by
2 Rubarth in the late 1940s, who identified transient corneal edema associated with infectious
3 canine hepatitis caused by canine adenovirus type 1 (CAV-1).¹ Curtis and Barnett in 1983
4 found corneal edema occurred as a result of administration of modified live CAV-1 vaccines,
5 and calculated an overall incidence of 0.4%.² This association resulted in CAV-1 vaccines
6 being replaced with attenuated CAV-2 based vaccines, which had good cross-protective
7 immunity but did not appear to cause keratouveitis.³

8 Canine adenoviruses belong to the genus *Mastadenovirus* of the family Adenoviridae.⁴ In
9 dogs, CAV-1 causes infectious canine hepatitis (ICH) while CAV-2 causes canine infectious
10 laryngotracheitis. ICH can present clinically with signs of pyrexia, inappetence, lethargy,
11 vomiting, diarrhea, conjunctivitis, petechial hemorrhages, tachypnea, corneal edema and
12 rarely icterus or neurological signs.⁵ Keratouveitis has been observed in approximately 20%
13 of dogs recovering from overt ICH clinical infection but has also been described in other
14 diseases such as leishmaniasis.^{1,6} The corneal edema observed usually occurs one to three
15 weeks after infection with CAV-1 and develops because of a type III immune-complex
16 hypersensitivity reaction within the eye. Antigen-antibody complexes are deposited on the
17 corneal endothelium causing damage to these cells.²

18 Confirmation of a diagnosis of CAV infection can be based on virus isolation, PCR,
19 histopathology, and serological tests. Virus neutralizing testing has been described as the
20 ‘gold standard’ method for the detection of antibodies against CAV.⁷ Serum neutralization
21 tests provide a retrospective diagnosis, and their interpretation can be challenging when
22 recent vaccinations have been used. Titers that follow natural infection may be higher than
23 those that follow vaccination.⁵ Research also suggested that a four-fold rise in titer over 2-3

1 weeks (a convalescence titer) along with clinical signs can be supportive of a diagnosis of
2 infectious canine hepatitis.⁵

3 In the present study, we reviewed clinical data associated with cases of suspected
4 keratouveitis collected at the Animal Health Trust over a ten-year period. To evaluate the
5 potential role of CAV as a causal agent, we measured CAV-1 and CAV-2 antibody titers in
6 affected dogs and control cases using serum neutralization assays. To the authors knowledge
7 no study to date has correlated CAV titers with keratouveitis.

8 **Materials and Methods:** A review of the medical records of every dog that presented to the
9 Animal Health Trust ophthalmology service over a ten-year period, between 2008 and 2018,
10 was undertaken. Dogs less than one year old, presenting with sudden onset corneal edema
11 and anterior uveitis, with a known vaccination status, that underwent a full ophthalmic
12 examination and had CAV serology performed were included in the study. Dogs that had
13 other ocular pathologies that could cause corneal edema or uveitis, such as primary glaucoma
14 or corneal ulceration, were excluded.

15 Nine age-matched dogs without corneal edema but with stored blood from previous
16 investigations had CAV titers performed and were included in the study as controls. These
17 dogs all had known clinical diseases that were unrelated to canine adenovirus.

18 All CAV serology was performed at Glasgow university's veterinary diagnostics laboratory
19 on fresh or frozen serum samples. The serum neutralization testing for both CAV-1 and
20 CAV-2 that was performed involved four-fold dilutions of test serum being made in culture
21 medium in the range 1:4 to 1:16,384, with each dilution represented by four replicates. Stock
22 adenovirus (either CAV-1 (strain 47889) or CAV-2 (Manhattan strain)) and Madin-Darby
23 Canine Kidney (MDCK) cells were added to each well and, following incubation, plates were

1 examined microscopically for a cytopathic effect. Antibody titers were calculated from the
2 endpoints across all four replicates using the Spearman-Kärber method.

3 Statistical analysis was performed to compare the affected dogs' CAV titers with those of the
4 controls using a t-test for two values assuming unequal variance, with statistical significance
5 set at $p < 0.05$.

6 Ethical approval was attained through the Animal Health Trust ethical approval committee
7 Project number and investigator: 43-2018 Hannah Joyce.

8 **Results:** Nine dogs met the inclusion criteria, representing a total of 14 affected eyes. All
9 dogs presented with corneal edema which ranged from mild to marked and focal to pan-
10 corneal (see figures 1 and 2). Eight of the nine dogs had signs of anterior uveitis and seven
11 had keratic precipitates present. Eight different breeds were represented.

12 Five dogs developed keratouveitis within one month of CAV-2 vaccination. The age range
13 was 2-11 months, with a mean of 4.8 months and a median of 3 months. Four different
14 brands of vaccine containing live attenuated CAV-2 were used, namely Nobivac (MSD),
15 Canigen (Virbac), Duramune (ELANCO) and Procyon (CMS).

16 The corneal edema resolved in four dogs following treatment. Three resolved with medical
17 management using both topical and systemic anti-inflammatory medications (detailed in table
18 1.) and in one case surgical treatment with thermal keratoplasty was elected. The corneal
19 edema did not resolve in two dogs at the last examination (two- and seven-months post-
20 testing respectively) and two dogs were lost to follow-up. Two dogs required enucleation of
21 one eye due to the development of secondary glaucoma.

1 Further information on the nine keratouveitis cases including the sex, CAV-1 and CAV 2
2 serology titer results, diagnostic tests that were performed and the other clinical signs that
3 were observed are presented in table 1.

4 The CAV-1 and CAV-2 titers were not statistically different between dogs with keratouveitis
5 and age-matched controls ($p=0.16$ and $p=0.76$, respectively). This indicates that exposure to
6 either CAV-1 or CAV-2 cannot fully account for the keratouveitis. However, three of the
7 blue eye cases had markedly high CAV-1 titers ($>5,000$), each substantially greater than the
8 corresponding CAV-2 titers ($>four\text{-fold}$ difference). Two of these cases had convalescence
9 titers performed 20-23 days later which had markedly increased CAV-1 titers (256 that
10 increased to 5792 [>20 fold increase] and 1448 that increased to 16381 [>11 fold increase])
11 which is indicative of infection with wild-type CAV-1. The CAV titers in the six other cases
12 were not highly indicative of either CAV-1 or CAV-2 infection. Two dogs were
13 unvaccinated; one had titers consistent with protection for CAV (CAV-1: 1,448 and CAV-2:
14 512) while the other dog had very low titers (CAV-1: 32 and CAV-2: 11) suggesting it was
15 not protected against this virus and it had not been exposed to CAV in the past.

16 **Discussion:** Our study confirms keratouveitis still occurs despite the advent of CAV-2 based
17 vaccination. Wild-type CAV-1 infection should be considered as a possible differential when
18 presented with a young dog with keratouveitis. No evidence was found to indicate that the
19 CAV-2 vaccine component can cause this anomaly.

20 One study examined serum neutralization titers in 144 adult dogs unvaccinated for at least
21 three years and compared them to 199 puppies after they had received their initial
22 vaccinations; dogs with CAV titers below 16 were considered susceptible to disease. Those
23 with titers between 16-64 were considered to be possibly protected and those with titers
24 above 64 were almost certainly protected.⁸ Bergmann et al found, in vaccinated dogs, the

1 highest recorded titer for CAV-1 to be 2,560 and titers for CAV-2 to not rise above 1280.⁹
2 Titres above 256 were found to be associated with repeated vaccination or natural CAV
3 infections.¹⁰ Three out of the nine keratouveitis dogs in this study had CAV-1 titers greater
4 than 2,560 and CAV-2 titers greater than 1,280, however, none of the controls had titers
5 above 724 for CAV-1 and only one had a CAV-2 titer greater than 1,280. This suggests the
6 keratouveitis cases had increased exposure to CAV in comparison to the controls.

7 Keratouveitis can be caused by a multitude of conditions, both intraocular and systemic,
8 including infectious and non-infectious causes. In the review by Massa et al of the causes of
9 canine uveitis in North America, in young dogs the most common underlying cause was
10 infectious diseases (17.6%).¹¹ The present study was performed in the United Kingdom
11 where there are comparatively fewer infectious diseases (most markedly mycotic diseases)
12 causing anterior uveitis in the dog and so testing for infectious diseases was limited.⁶
13 However, we cannot exclude infectious agents (and others) as potential underlying causes.
14 Idiopathic uveitis tends to occur in middle-aged dogs and neoplastic causes are considered
15 less likely in younger dogs, although lymphoma has been reported to occur in dogs less than
16 one year of age.¹² These varied causes mean that a full systemic work-up is warranted in
17 cases that present with keratouveitis.

18 Limitations of this study include the low case number due to the sporadic nature of this
19 condition, the inconsistency and lack of diagnostic tests that were performed (due to the
20 retrospective nature of the study) and that convalescence titers were only performed in three
21 cases. Further studies with larger case numbers undergoing full systemic work-up, to include
22 testing for other infectious diseases as well as repeated CAV titres, could be beneficial in
23 determining the underlying causes of keratouveitis. With the titer results of three of our dogs

1 suggestive of CAV-1 as the underlying cause of keratouveitis, CAV serology remains a
2 useful diagnostic test in young dogs with this condition.

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19 Conflict of Interest statement: The authors have no conflict of interests to declare

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