AUSTRALIAN VETERINARY JOURNAL
AUSTRALIA'S PREMIER VETERINARY SCIENCE TEXT
SMALL ANIMALS

SHORT COMMUNICATION

Update on feline alphaherpesvirus-1 seroprevalence in Victorian feral and owned cats

E Cottingham,[a]* T Johnstone,[b] CA Hartley[a] and JM Devlin[a]

The seroprevalence of feline alphaherpesvirus-1 (FHV-1) in feral cats in Victoria, Australia, was last assessed in 1981 when serum-virus-neutralising antibodies (VNAb) against FHV-1 were detected in 11% of the sampled population from two Victorian locations. In this current study, VNAb were assessed in serum from feral cats located in Phillip Island, Point Cook and Hattah in the Mallee region in Northern Victoria. In feral cats, the seroprevalence of VNAb to FHV-1 was highest in Point Cook at 24.6% (17/69), followed by Phillip Island at 16.7% (11/66) and Hattah where no feral cats had detectable VNAb to FHV-1 (0/12). In contrast, virus-neutralising antibodies were observed in 84.1% (37/44) of Victorian-owned cats. This higher seroprevalence in owned cats is likely due to the use of FHV-1 vaccines; however, the vaccination history of the cats was not known and the development of neutralising antibodies after infection or vaccination can vary. The results are useful for understanding FHV-1 exposure in feral and owned cats and are important background information in the context of any potential future use of FHV-1-vectored vaccines.

Keywords feline herpesvirus; feral cats; seroprevalence

Abbreviations FHV-1, feline alphaherpesvirus-1; VNAb, virus-neutralising antibodies

Aust Vet J 2022;100:187–189 doi: 10.1111/avj.13147

Feline alphaherpesvirus-1 (FHV-1) is a common cause of upper respiratory tract disease in cats worldwide.[1] In a 1981 study, 11% (14/123) of feral cats demonstrated neutralising antibodies against FHV-1 in serum-virus-neutralisation assay when surveyed over two sites in the Mallee and North Central Victoria.[2] An absence of FHV-1 serological surveys in Victorian feral cats since that time has meant that there have been no opportunities to update this information. Examining FHV-1 seroprevalence in feral cats can help to assess infectious risks that feral cats may pose to pet (owned) cats. In addition, understanding FHV-1 exposure in both feral and owned cats may be helpful in the context of any future development and use of vaccines that utilise FHV-1 as a vector, including FHV-1-vectored immunocontraceptive vaccines that have been proposed as a tool to help control feral cat populations.[3, 4]

To determine the proportion of feral cats exposed to FHV-1 in 2021, 40 years since the first study, serum samples from three separate populations of feral cats in Victoria were assessed for FHV-1-neutralising antibodies. Serums were collected from cats in Hattah (12 cats), Point Cook (69 cats) and Phillip Island (66 cats) (Figure 1). Samples from Phillip Island and Hattah were collected by Parks Victoria, Mallee Central Catchment Authority and Department of Environment, Land, Water and Planning personnel as part of ongoing feral cat management strategies. Samples from Point Cook were collected by the Wyndham City Council personnel. All serum samples were collected between mid-2016 and mid-2021 from feral cats postmortem after the animals were humanely culled for reasons unrelated to this study. Body weight was recorded from animals at Hattah and Phillip Island. In addition to samples from feral cats, serum samples from 44 owned (pet) cats were obtained from ASAP laboratories in Mulgrave, Victoria. These samples had been previously sent to ASAP laboratories from veterinary clinics around Victoria and on the Victorian-NSW border in 2019. The serum utilised in this study was excess to the requirements for laboratory testing by ASAP. Before their release from the ASAP laboratories, samples were de-identified and re-labelled numerically for our use, only the age of the cat was provided for each sample.

The serum-virus-neutralisation assay to assess the prevalence of VNAb in Victorian feral and owned (pet) cats was conducted at laboratories at the University of Melbourne using methods similar to those that we have previously described[5] and using FHV-1 (Companion Intervet Vaccine strain)[6] cultured in Crandell Rees feline kidney cells.[7] Briefly, serums were diluted to 1/5 before serial twofold dilutions of serums were performed to a final dilution of 1/640. The serums were incubated with 100 of 50% tissue culture infective dose (100 $TCID_{50}$) of FHV-1. The 96-well trays were incubated for 1 h at 37°C before the addition of Crandell Rees feline kidney cells and incubation for a further 3 days at 37°C in a humidified atmosphere of 5% v/v CO_2 in air. Each serum was tested in duplicate, and each assay included cell-only (no virus) control wells, and a virus titration to confirm 100 $TCID_{50}$ of virus was added in every assay. Antibody titers were recorded as the reciprocal of the highest dilution of the serum to neutralise virus. The antibody titers for samples where no viral neutralisation was observed at any dilution were recorded as <5.

*Corresponding author
[a]The Asia Pacific Centre for Animal Health | Melbourne Veterinary School, The University of Melbourne, Parkville, Victoria, 3010, Australia; ellenpc@student.unimelb.edu.au
[b]Animal Referral Hospital, Essendon Fields, Victoria, 3041, Australia
CA Hartley and JM Devlin contributed equally.

Table 1 shows the seroprevalence results in the different groups of cats. In feral cats, the seroprevalence results ranged from no evidence of FHV-1 VNAb in feral cats from Hattah, to the highest level of seroprevalence in feral cats from Point Cook, where 17 out of 69 samples (24.6%) had detectable FHV-1 VNAb. In owned cats, 84.1% (37/44) of cats had detectable FHV-1 VNAb (Table 1). While there was no significant difference between the proportion of seropositive cats between the three feral cat locations, all three feral cat groups showed significantly lower seroprevalence compared with the owned cats ($P < 0.0001$, Fisher's exact test). Neutralising antibody titers were not significantly different between owned and feral cats when compared using the Mann-Whitney U-test ($P > 0.05$). The weights of the cats from Phillip Island and the age of the owned cats were tested for any correlation with VNAb titer using Pearson's correlation and nonparametric Spearman's correlation, respectively. No correlation was observed in either instance ($P = 0.12$, $P = 0.54$). In general, the titers of neutralising antibodies were consistently low compared with the neutralising antibody titers than can be induced by some other alphaherpesviruses in their respective mammalian hosts.[8]

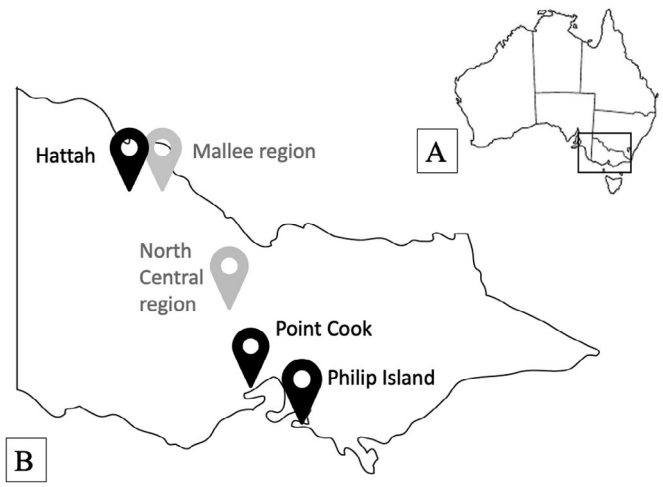

Figure 1. **(A) Map of Australia with Victoria outlined and (B) the outline of Victoria, Australia, showing the location of sampling sites from a previous 1981[2] study assessing the prevalence of serum antibodies to feline alphaherpesvirus-1 in feral cats 1981 (light markers) and the location of sampling sites in this present study (dark markers).**

The results from the feral cats in the current study reflect the 1981 findings by Coman et al where FHV-1 seroprevalence levels were low across the two sites; 9% in the Mallee region and 17% in the North-West central region of Victoria. The prevalence of neutralising antibodies in the owned cats in this study was high. Vaccination histories were not available, but the use of FHV-1 vaccines is common in owned Australian cats, and these vaccines are known to induce neutralising antibodies.[9, 10] Neutralising antibodies induced either by vaccination or infection with FHV-1 are broadly neutralising against all strains of FHV-1 due to the high homology and antigenic similarity of FHV-1 strains globally.[6, 11]

We were unable to find any previous records of neutralising antibody concentrations to FHV-1 in owned cats in Victoria, thus a comparison across different studies and years was not possible. It would be useful for future studies assessing FHV-1 neutralising antibodies in owned cats to also examine vaccination history if possible.

Felid alphaherpesvirus-1 is fragile in the environment, remaining infectious for up to 18 h in humid or damp conditions[12] and is typically spread by close-contact between cats in crowded settings such as in shelters.[13] It is likely that FHV-1 is transmitted both within and between owned and feral cat populations when these different populations come into contact. In regions with high FHV-1 seroprevalence in feral cats, it would be prudent to have a particular focus on vigilant FHV-1 vaccination of owned cats to help protect these cats from disease. Cats vaccinated against FHV-1 are generally protected against clinical disease but not infection with FHV-1[14] and thus may still play a role in the transmission and maintenance of FHV-1 infection in cat populations. The absence of detectable VNAb to FHV-1 in feral cats in the more remote Mallee region is noteworthy. It is possible this reflects a less dense population where FHV-1 is less readily spread, although the number of samples from this region was small and data relating seroprevalence of FHV-1 to the density of cat populations in Victoria are limited. Future studies with larger number of cats from the Mallee and other remote regions would be useful for defining FHV-1 seroprevalence more precisely, although this does have practical and logistical challenges. Future

Table 1. **Serum-virus-neutralisation assay results from testing samples from feral and owned cats in Victoria**

Cat population	Number of serum samples	Number (%) with FHV-1 VNAb titers ≥5	Median (range) antibody titer of positive samples	FHV-1 seroprevalence (%) 95% confidence interval	Clinical notes: Mean weight (kg ± SD) median age in years (range)
Point Cook (feral)	69	17 (24.6%)[a]	17.5 (<5–120)	16.0–36.0%	N/A
Phillip Island (feral)	66	11 (16.6%)[a]	20 (5–80)	9.6–27.4%	Mean weight = 2.8 ± 1.19 kg
Hattah (feral)	12	0 (0%)[a]	NA	0.0–24.3%	Mean weight = 4.7 ± 0.92 kg
Owned cats	44	37 (84.1%)[b]	15 (2.5–160)	70.6–92.1%	Median age = 12 years (1–19 years)

Seroprevalence (%) values with different superscript lettering were significantly different (Fisher's exact test). There were no significant differences in VNAb titers (Mann-Whitney U-test).
FHV-1, feline alphaherpesvirus-1; VNAb, virus-neutralising antibodies.

studies (in both owned and feral cats) could also assess exposure to additional feline pathogens.

FHV-1 has been investigated as a viral vector for the development of a range of different vaccines for owned cats and has been proposed as a vector for feline immunocontraceptive vaccines for both owned and feral cats.[3] The low levels of FHV-1-neutralising antibodies in feral cats may be advantageous for the future use of FHV-1-vectored immunocontraceptive vaccines, as high levels of vector replication, robust immune responses to vectored reproductive antigens and possible horizontal transmission between individuals could be facilitated in naïve animals. In contrast, a high seroprevalence of neutralising antibodies in domestic/owned cats, likely originating from prior FHV-1 vaccination, indicates that any FHV-1-vectored vaccines would need to be efficacious in animals with prior FHV-1 exposure if they were to be useful for this population of animals.

Acknowledgments

We thank staff from Parks Victoria, Mallee Central Catchment Authority, Department of Environment, Land, Water and Planning and Wyndham City Council for collecting samples for us as part of their feral cat management strategies. We thank Dr Alan Robley at Arthur Rylah Research Institute for his help with coordinating sample collection. ASAP laboratories are acknowledged for allowing access to discarded serum samples, and we thank A/Prof Natalie Courtman (Melbourne Veterinary School) for helping to facilitate this. We thank Dr Andrew Stent for his helpful advice on postmortem collection of blood samples.

Open access publishing facilitated by The University of Melbourne, as part of the Wiley - The University of Melbourne agreement via the Council of Australian University Librarians.

Conflict of interest and sources of funding

The authors declare no conflicts of interest or sources of funding for the work presented here.

References

1. Maes R. Felid herpesvirus type 1 infection in cats: a natural host model for alphaherpesvirus pathogenesis. *ISRN Vet Sci* 2012;2012:1–14. https://doi.org/10.5402/2012/495830.
2. Coman BJ, Jones EH, Westbury HA. Protozoan and viral infections of feral cats. *Aust Vet J* 1981;57:319–323. https://doi.org/10.1111/j.1751-0813.1981.tb05836.x.
3. Cottingham E, Johnstone T, Hartley CA et al. Use of feline herpesvirus as a vaccine vector offers alternative applications for feline health. *Vet Microbiol* 2021;261:109210. https://doi.org/10.1016/j.vetmic.2021.109210.
4. Goericke-Pesch S, Wehrend A, Georgiev P. Suppression of fertility in adult cats. *Reprod Domest Anim* 2014;49:33–40.
5. Warner S, Hartley CA, Stevenson RA et al. Evidence that equine rhinitis a virus VP1 is a target of neutralizing antibodies and participates directly in receptor binding. *J Virol* 2001;75:9274–9281. https://doi.org/10.1128/jvi.75.19.9274-9281.2001.
6. Vaz PK, Job N, Horsington J et al. Low genetic diversity among historical and contemporary clinical isolates of felid herpesvirus 1. *BMC Genomics* 2016;17:704. https://doi.org/10.1186/s12864-016-3050-2.
7. Crandell RA, Fabricant CG, Nelson-Rees WA. Development, characterization, and viral susceptibility of a feline (Felis catus) renal cell line (CRFK). *In Vitro* 1973;9:176–185.
8. Goodman LB, Wimer C, Dubovi EJ et al. Immunological correlates of vaccination and infection for equine herpesvirus 1. *Clin Vaccine Immunol* 2012;19:235–241. https://doi.org/10.1128/CVI.05522-11.
9. Lappin MR, Andrews J, Simpson D et al. Use of serologic tests to predict resistance to feline herpesvirus 1, feline calicivirus, and feline parvovirus infection in cats. *J Am Vet Med Assoc* 2002;220:38–42.
10. Johnston L, Szczepanski J, McDonagh P. Demographics, lifestyle and veterinary care of cats in Australia and New Zealand. *J Feline Med Surg* 2017;19:1199–1205.
11. Horimoto T, Limcumpao JA, Xuan X et al. Heterogeneity of feline herpesvirus type 1 strains. *Arch Virol* 1992;126:283–292. https://doi.org/10.1007/BF01309701.
12. Gaskell R, Dawson S, Radford A et al. Feline herpesvirus. *Vet Res* 2007;38:337–354. https://doi.org/10.1051/vetres:2006063.
13. DiGangi BA, Levy JK, Griffin B et al. Prevalence of serum antibody titers against feline panleukopenia virus, feline herpesvirus 1, and feline calicivirus in cats entering a Florida animal shelter. *J Am Vet Med Assoc* 2012;241:1320–1325. https://doi.org/10.2460/javma.241.10.1320.
14. Thiry E, Addie D, Belák S et al. Feline herpesvirus infection: ABCD guidelines on prevention and management. *J Feline Med Surg* 2009;11:547–555. https://doi.org/10.1016/j.jfms.2009.05.003.

(Accepted for publication 6 January 2022)