



# Optimizing the detection of persistent Amdoparvovirus in zoo-housed Red Pandas (Ailurus fulgens)



**Optimizing the detection of persistent Amdoparvovirus in zoo-housed Red Pandas (Ailurus fulgens)**  
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**Objectives**


- Develop and validate a qPCR assay for Red Panda Amdoparvovirus (RPV).
- Quantify shedding of RPV in serial fecal samples from persistently infected red pandas.
- Identify historical cases of RPV by qPCR testing of formalin-fixed, paraffin-embedded (FFPE) tissue sections.

**Methods and Results**

**Fig. 2.** (A) An RPV genome sequence including the 123-nt qPCR target was inserted into a plasmid vector, and qPCR of serial dilutions was used to generate a standard curve. This was used to quantitate viral copy numbers in fecal samples (B) and to analyze, formalin-fixed, paraffin-embedded (FFPE) tissues (C).



**Fig. 3A&B.** RPV was consistently shed in serial fecal samples of two asymptotically infected red pandas over a study period of 75 days.



**Conclusions**

**qPCR**  
 We adapted and validated a qPCR assay for detection of RPV. The assay is sensitive, capable of detecting as few as three template DNA copies in a sample, and is effective for amplification from fecal and tissue (FFPE) samples. This is a critical tool with many potential applications in our efforts to understand the biological behavior, epidemiology, and pathogenesis of RPV.

**Background and Significance**

Persistent (life-long) Amdoparvovirus (APV) infections are common in many wild mammal species, with a spectrum of clinical manifestations ranging from asymptomatic to fatal disease. Our lab discovered a novel APV infecting zoo-housed red pandas (*Ailurus fulgens*), and we recently established that infections are highly persistent (90%) in this population. Host-specific APVs can be pathogenic in other species, so the high prevalence of RPV in this endangered species is concerning, and while most infected red pandas appear to be clinically normal, pilot studies of tissue distribution have shown that RPV can be associated with significant inflammatory lesions (Fig. 1).

The work presented here was supported by the National Institutes of Health (NIH).

**Future Directions and Acknowledgements**

**Future Directions:**  
 More research is required to fully characterize the behavior of RPV, such as discovering the age at which red pandas begin to shed virus, and whether shedding will increase with age. Characterizing rates and patterns of viral evolution is also critical for evaluating the potential emergence of novel genotypes with increased virulence or altered host tropism. Additionally, to get a better grasp on its epidemiology, we must answer if there is individual variation of RPV within a single red panda or between members of a cohort, and if there is a difference in viral

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PRESENTED AT:



## OBJECTIVES

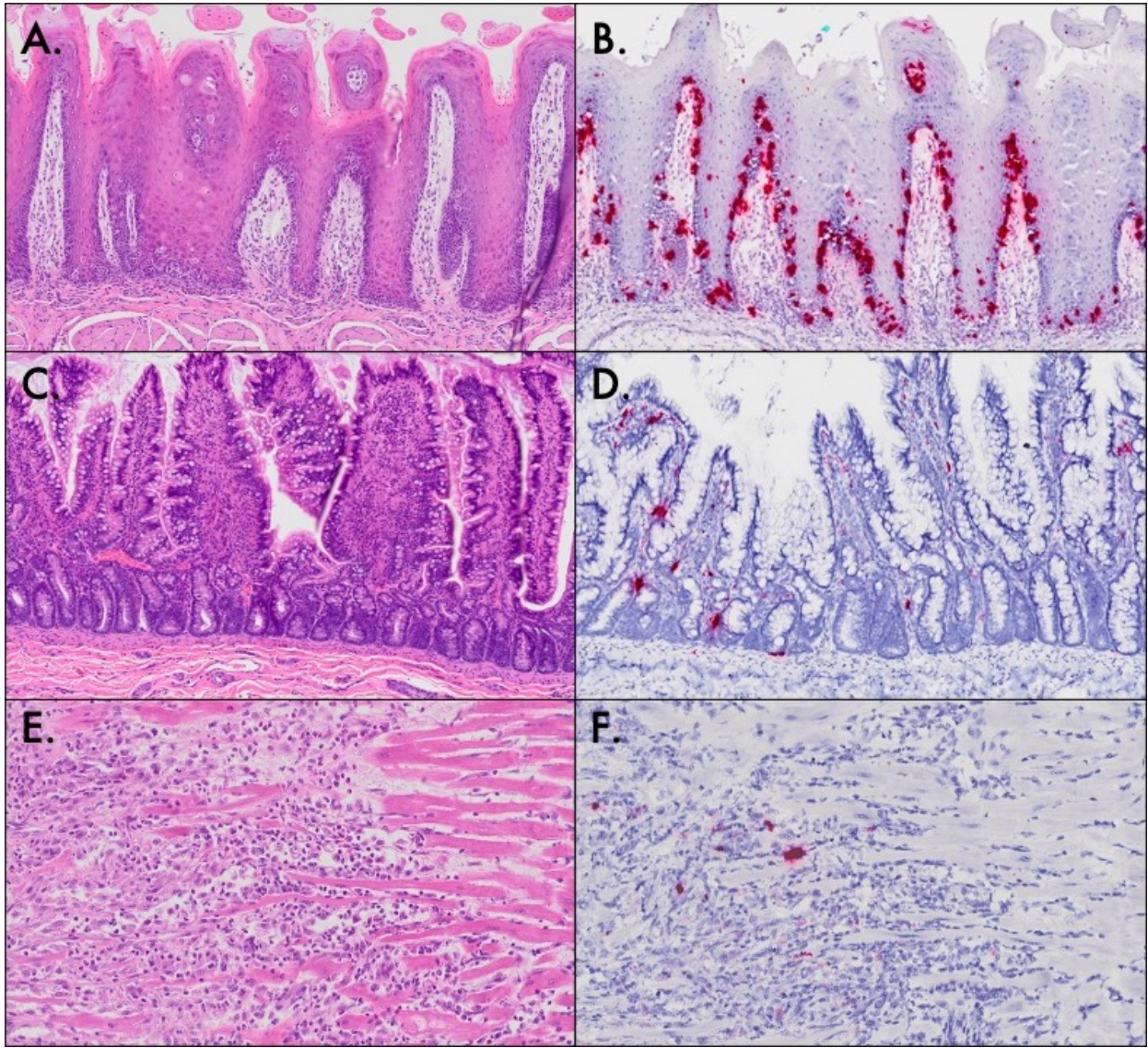
1. Develop and validate a qPCR assay for Red Panda Amdoparvovirus (RPAV).
2. Quantitate shedding of RPAV in serial fecal samples from persistently infected red pandas.
3. Identify historical cases of RPAV by qPCR testing of formalin-fixed, paraffin-embedded (FFPE) tissue scrolls.

## BACKGROUND AND SIGNIFICANCE

Persistent (life-long) Amdoparvovirus (APV) infections are common in many carnivore species, with a spectrum of clinical manifestations ranging from asymptomatic to fatal disease.<sup>1-4</sup> Our lab discovered a novel APV infecting zoo-housed red pandas (*Ailurus fulgens*)<sup>5</sup>, and we recently established that infections are highly prevalent (50%) in this population. Host-specific APVs can be pathogenic in other species<sup>6,7</sup>, so the high prevalence of RPAV in this endangered species is concerning, and while most infected red pandas appear to be clinically normal, pilot studies of tissue distribution have shown that RPAV can be associated with significant inflammatory lesions (Fig. 1).

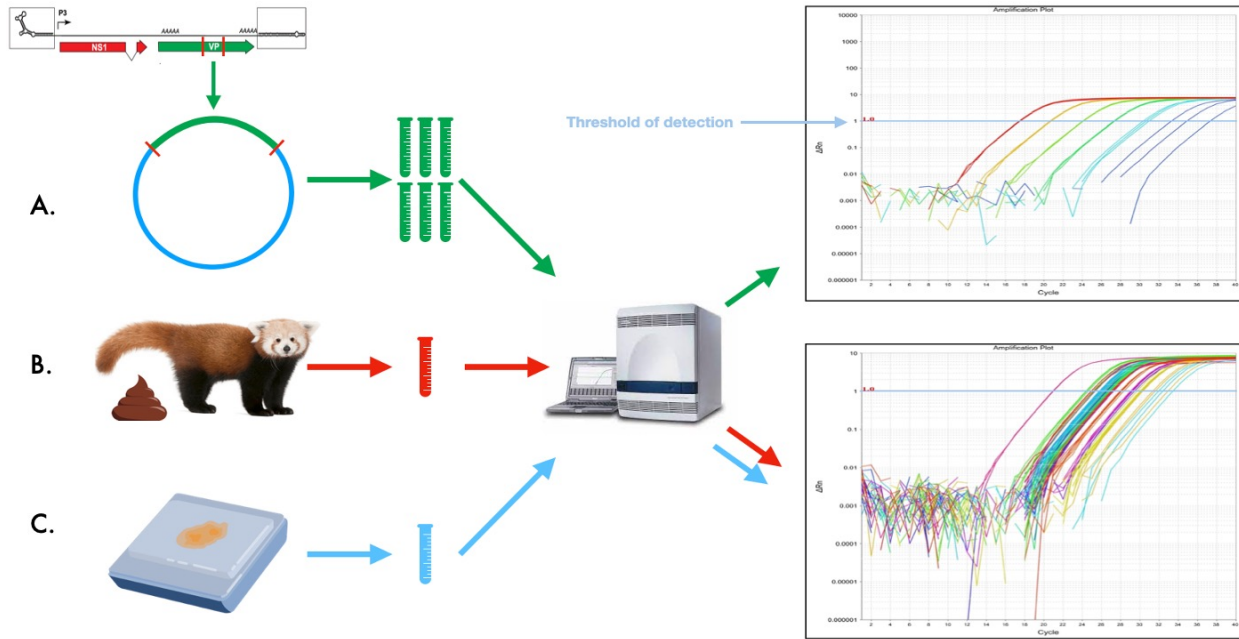
To understand the population-level health impact of RPAV in red pandas, we need to understand its natural history, epidemiology, biological behavior, determinants of virulence, and spectrum of pathologic manifestations. An accurate, sensitive, and reliable method of detection is critically needed for all of these efforts, and will be a powerful tool in determining the significance of these infections in this endangered species.

**Fig. 1.** RPAV is consistently detected in lingual (A, B) and intestinal (C, D) epithelium, which are suspected to be sites of persistence and sources of fecal shedding. RPAV has also been demonstrated in association with inflammatory lesions, including myocarditis (E, F).

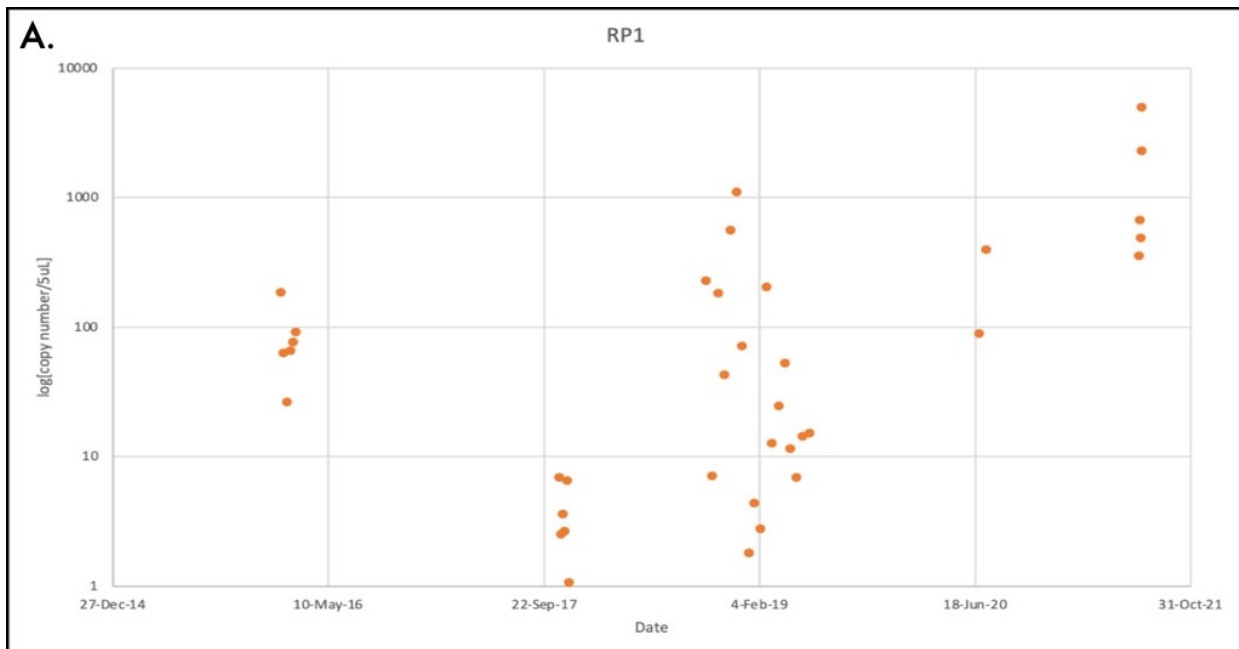


## METHODS AND RESULTS

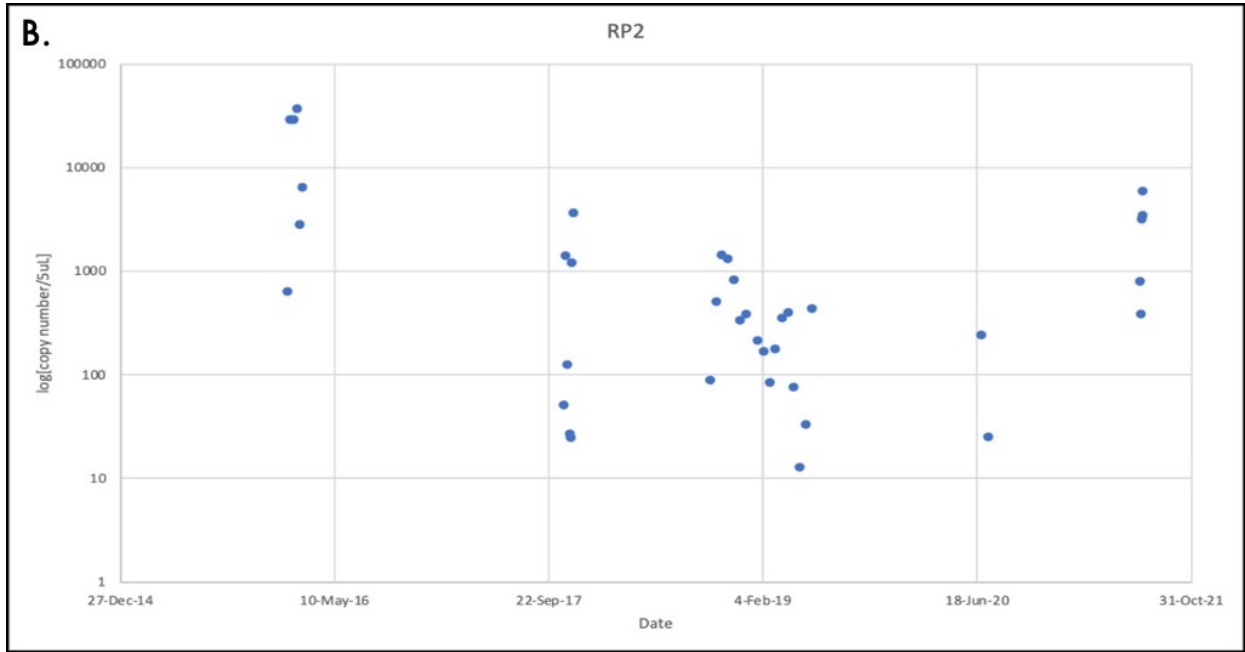
**Fig. 2. (A)** An RPAV genome segment including the 121-nt qPCR target was inserted into a plasmid vector, and qPCR of serial plasmid dilutions was used to generate a standard curve. This was used to quantitate viral copy numbers in fecal samples **(B)** and in archived, formalin-fixed, paraffin-embedded (FFPE) tissues **(C)**.



**Fig. 3A&B.** RPAV was consistently shed in serial fecal samples of two asymptotically infected red pandas over a study period of >5 years.







## CONCLUSIONS

### **qPCR**

We adapted<sup>8</sup> and validated a qPCR assay for detection of RPAV. The assay is sensitive, capable of detecting as few as three template DNA copies in a sample, and is efficient for amplification from fecal and tissue (FFPE) samples. This is a critical tool with many potential applications in our efforts to understand the biological behavior, epidemiology, and pathogenesis of RPAV.

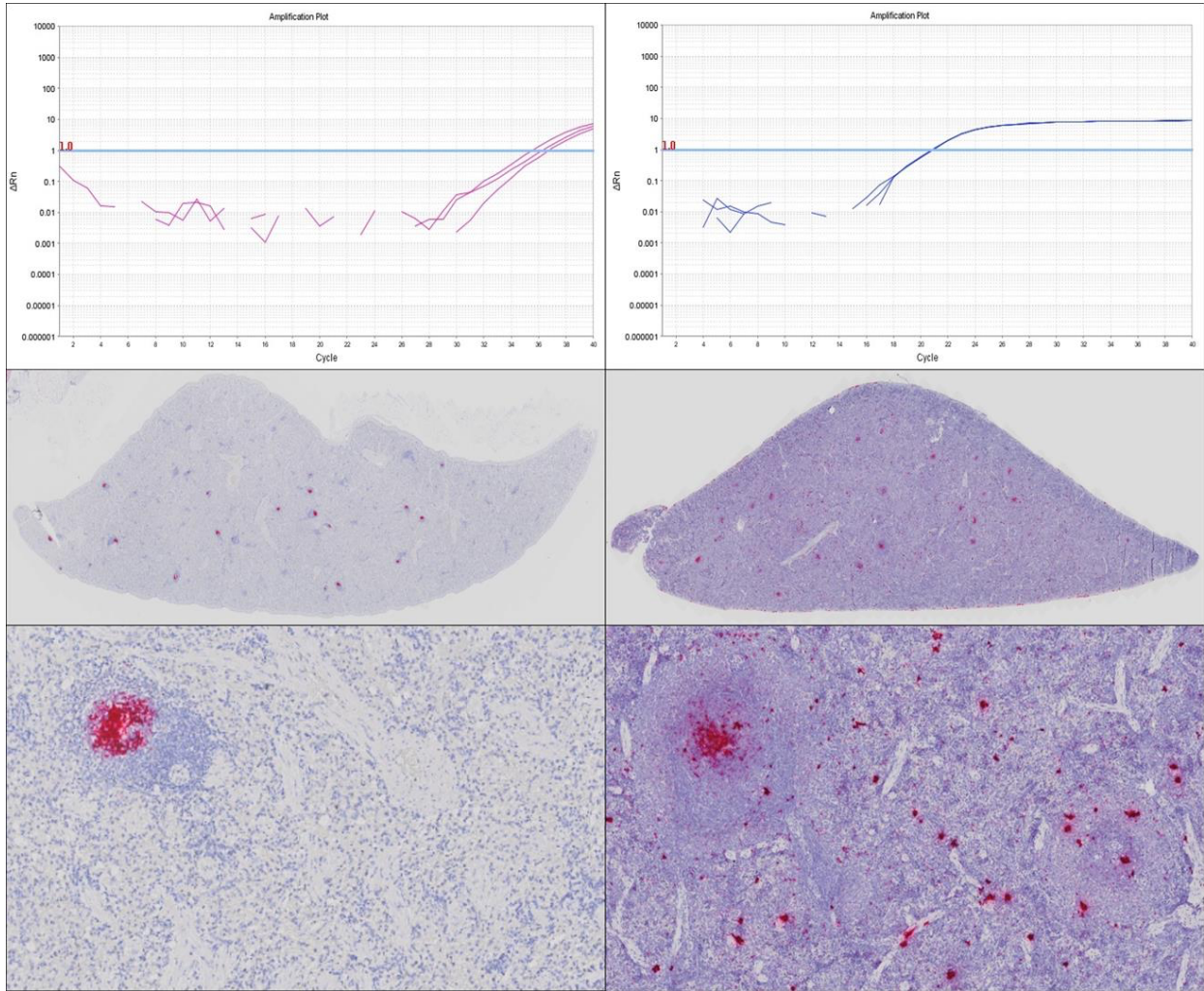
### **Characterization of fecal shedding**

Asymptomatically infected animals consistently shed RPAV in feces for the duration of the study period (January 2016 – August 2021). This is a key insight into the biology of RPAV, suggesting that transmissions could occur during any period of close contact (co-housing). Quantifying virus in fecal samples also facilitates sequencing efforts by allowing for targeting of samples with high viral loads. This will support ongoing objectives to characterize viral evolution (rates and patterns of change across longitudinal samples) and molecular epidemiology (dissemination of viral lineages throughout the zoo-housed population).

### **Detection of RPAV in archived cases**

We validated the qPCR assay for use with FFPE tissues. This sensitive method to detect RPAV in tissue samples will allow us to identify archived cases for studies of tissue distribution and associated lesions by in situ hybridization. Uncovering the spectrum of RPAV-associated disease is critical in understanding the pathogenesis and impact of these infections.

**Fig. 4.** qPCR of DNA extracted from FFPE tissue samples successfully detected RPAV in archived pathology cases, and viral load (CT) is subjectively correlated with intensity of signal detected by ISH.





## FUTURE DIRECTIONS AND ACKNOWLEDGEMENTS

### **Future Directions:**

More research is required to fully characterize the behavior of RPAV, such as discovering the age at which red pandas begin to shed virus, and whether shedding will increase with age. Characterizing rates and patterns of viral evolution is also critical for evaluating the potential emergence of novel genotypes with increased virulence or altered host tropism. Additionally, to get a better grasp on its epidemiology, we must answer if there is individual variation of RPAV within a single red panda or between members of a cohort, and if there is a difference in viral load between the two red panda subspecies (Himalayan and Chinese). Lastly, to understand of the pathogenesis of RPAV, it is absolutely critical to determine tissue distribution in health and disease, and whether viral load is correlated with disease.

### **Acknowledgements:**

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## AUTHOR INFORMATION

Eric Stubbs is a rising 3rd year veterinary student at UC Davis. He is tracking small animal, and has broad interests that span the entirety of medicine, including wildlife, zoo, internal medicine, exotics, critical care, and surgery. If you have any questions regarding his research project, please contact him at [elstubbs@ucdavis.edu](mailto:elstubbs@ucdavis.edu).

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