Developing tools to investigate CDV in wild tigers

Contents

Background on the project

Canine Distemper Virus (CDV) poses a serious and growing threat to wild tigers and leopards, with confirmed infections across multiple populations. Understanding exposure levels and identifying potential reservoir species are crucial for effective disease mitigation, especially given the small and isolated nature of many tiger populations.

WVI has played a key role in advancing the understanding of CDV in wild carnivores. This has included advocating for surveillance, supporting diagnostic capacity development, and co-funding training workshops. In 2022, WVI partnered with Cornell University to establish serum neutralisation tests (SNTs) in Indonesia, Thailand, and Nepal. These were successfully used to screen archived samples and confirmed CDV exposure in both tigers and leopards.

The current method for detecting CDV antibodies—the SNT—is accurate but highly specialised, requiring live virus and advanced lab facilities. Only four of the ten tiger range states have in-country capacity to run SNTs, limiting widespread surveillance.

This project, set up in collaboration with researchers at the University of Kent, aimed to address these challenges by developing a novel assay for CDV. The planned approach was to utilise universal antibody-binding proteins (A and G), allowing testing across multiple wildlife species. The aim was an accessible bench top assay that could be used in local laboratories, without substantial investment.

Partners and Personnel Involved

Partners and personnel involved in developing the assay

Wildlife Vets International (**Olivia Walter**) secured funding from Wildcats Conservation Alliance, oversaw the management of the project and worked with the University of Kent and other partners to deliver on the project aims.

University of Kent led the development of the assay, conducting all laboratory studies.

Dr. Dave Beal (DB), from the School of Biosciences, led and supervised the initial research into the development of a non-species-specific ELISA for antibodies against canine distemper virus. The laboratory research was carried out by two MSc students, **Grace Lelliot** (GL) and **Charlotte Dalzell** (CD), under the supervision of Dr. Beal and **Prof. Mark Smales** (MS). The department had recently been recognised as a national "Centre for Advanced Diagnostics Development and Analysis" and had extensive experience both in developing novel assays and collaborating with industry partners to bring them to market.

Dr Jess Bodgener (JB), Durrell Institute of Conservation and Ecology, has a long history of investigating CDV in wild tigers. Dr Bodgener is coordinating a larger project investigating the health of Asian leopards and tigers in conflict situations. She provided context and guidance and assisted with planning and reporting.

University of Cornell: The Cornell K. Lisa Yang Center for Wildlife Health is dedicated to developing and implementing proactive, science-based solutions to address wildlife health. They will provided guidance and contributed to the evaluation of the project's success.

Dr Martin Gilbert (MG) is a leading expert in carnivore health, and was instrumental in establishing SNT for CDV in Thailand, Indonesia and Nepal. He has a wealth of experience overseeing and managing wildlife health surveillance programs and has been working with colleagues in Asia for more than 25 years. He provided context and guidance and assisted with planning and reporting.

Partners and personnel who will be involved in validating the assay *(this work has not yet begun)*

Initial testing of the assay will take place in the UK, using samples held by UK zoological collections. An agreement is in place with **Aspinall Foundation**, where we will be working with **Dr Jane Hopper**.

Following on from trials in the UK we will move to field testing in tiger rang states, Indonesia, Malaysia and Nepal. International partners involved in validating the assay are **Dr Bongot Mulia** at **Taman Safari Indonesia**, **Dr Farina Mustaffa Kamal** at **University Putri Malaysia** and **Dr Amir Sadaula** at **National Trust for Nature Conservation**.

The distribution of test kits and analysis of the resulting data will be overseen by **DB, JB** and **MG**.

Project goals, activities and outcomes to date

Project goal:

The goal of the project is to develop a novel ELISA test for antibodies to CDV, that is suitable for use in tigers and wild carnivores.

The assay will be validated by comparison to the gold-standard SNT using a minimum of 100 archived samples, from a range of species. Validation will take place both at Kent, and at field sites in Malaysia and Nepal.

By December 2025 we will have developed the assay and begun validation and field trials.

Testing will be complete by June 2026 and results will be submitted for publication in an appropriate open access peer-reviewed journal by December 2026.

Summary of current status and proposed next steps:

Initial work on developing the assay has been promising, with a proof of concept for the protein A/G marking and the identification and production of suitable recombinant antigens.

The development of the ELISA has suffered from poor signal strength, but this seems to be less of a problem when using dot blot technology.

Further work is needed to get the final assay, whether that be an ELISA, dot blot or lateral flow to the point of validation, however MSc students GL and CD have now completed their studies and are no longer available to work on this.

There are funds remaining in the budget, however these are currently allocated for validation of the assay, meaning further funds will need to be sought to be able to complete this work in full.

Details of planned activities, outputs and outcomes to date

Planned activity 1**:** the laboratory at the University of Kent will test a small number of protein constructs (<6) for their effectiveness in an ELISA assay when combined with Protein A/G

Planned output 1: six protein constructs will have been tested for their effectiveness in detecting commercially available mouse CDV antibodies in combination with Protein A/G.

Indicator 1: a small number of protein constructs will have been identified that could potentially be used in an ELISA.

Expected completion date: 30/04/25

Actual completion date: lab work was completed on 26/07/2025 and the MSc reports were submitted on 15/09/25.

Outcome: **Success**.

Protein generation

Based on a further review of the literature, the six protein constructs originally identified in the proposal, were reduced to four that warranted further investigation. All four were from the hemagglutinin spike protein. The sequence for the hemagglutinin spike protein was divided into four sections, named 1,2,3 and 4 (Chan et al., 2009). The protein constructs investigated were different assemblages of these sections as follows: 1+2+3+4, 2+3+4, 3+4 and 4. The resulting proteins were named accordingly, i.e. 1+2+3+4CDVp, 2+3+4CDVp etc..

The larger proteins, 1+2+3+4CDVp and 2+3+4CDVp proved more difficult to produce and efforts were further narrowed to focus on 3+4CDVp and 4CDVp. Both of these proteins were successfully produced using bacterial protein expression cells followed by inclusion body purification. This process generated proteins of reasonable purity which were confirmed to be the correct protein by MALDI analysis.

Preliminary ELISA trials

Due to the cost of the anti-CDV antibodies ELISA trials were undertaken using anti-His tag antibodies which will target the poly Histidine tag at the end C-terminus of both 3+4CDVp and 4CDVp. Signal was only detected at very high concentrations of antibody (1 in 10000) with a rapid fall off as the concentration fell. However, more promising results were achieved using “Dot blot” technology.

Dot blot trials

This method was investigated as alternative to the ELISA approach. In this instance, serial dilutions of of proteins 3+4CDVp and 4CDVp were placed onto nitrocellulose membranes and detected with anti-His tag antibodies, anti-CDV antibodies and CDV+ve serum. This approach proved successful as illustrated below.

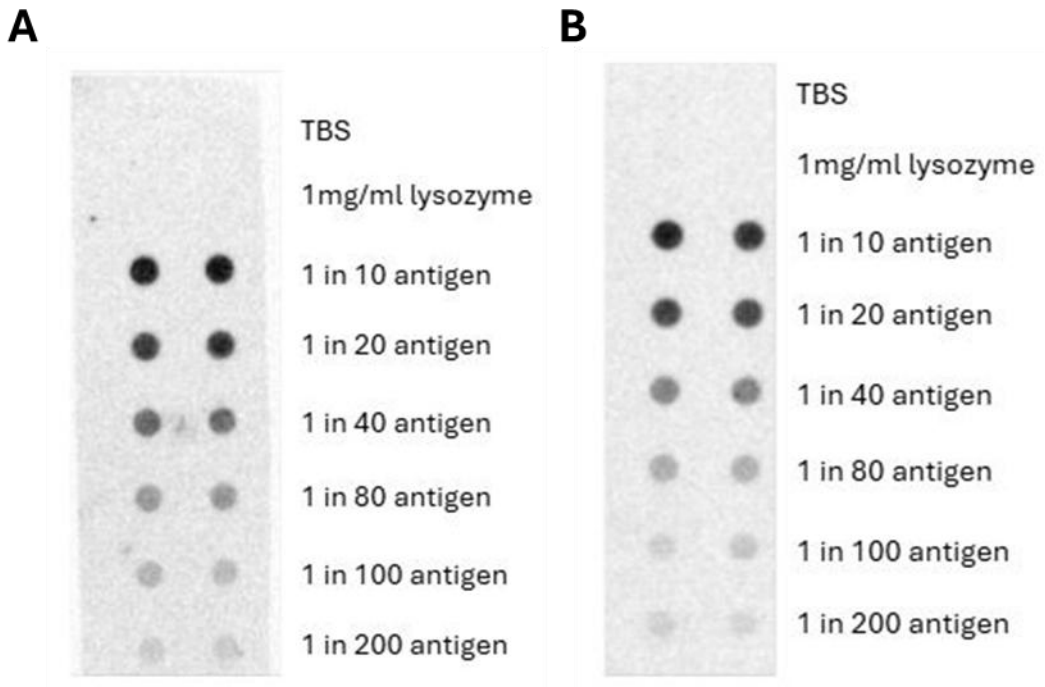

Fig. 1 Dot blot using 4CDVp which was detected by anti-CDV antibodies and CDV+serum samples. **A** Serial dilution of recombinant antigen detected with anti-CDV antibodies (1:2000 dilution) and detected using a mixture of Protein A/G HRP conjugates (1:10 dilution. **B** As A but anti-CDV antibodies replaced with CDV+ve serum (1:1000 dilution).

Investigating Proteins A and G as non-species specific a marker

Bacterial proteins A and G, which bind to antibodies from a large number of species antibodies, were modified with horse radish peroxidase (HRP) to give signal. The positive results from the assay in figure 1 mean that Protein A/G are an excellent tool to detect antibodies in assays of this type moving forward.

Planned activity 2: Further laboratory-based testing of a few protein constructs to enable the choosing of the final protein construct.

Planned output 2: Preliminary ELISA assay studies will be complete.

Indicator 2: we will have the final protein construct that can be deposited onto an ELISA plate and used to test for the presence/absence of CDV antibodies.

Expected completion date: 30/05/25

Actual completion date: Not yet completed.

Outcome: Partial success

Further investigations were carried out into alternative testing modalities including a lateral flow. The underlying principle of dot blot assays and lateral flow assays are similar in that the antigen is added to a membrane, blocked and the sample is probed by a mixture of primary and secondary antibodies. Typically a detection system utilising gold nanoparticles, AuNP, is utilised

for lateral flow assays giving them an easily discernible red band when a positive interaction is present.

Lateral flow trials

The protein samples were added to Vivid nitrocellulose membranes and then incubated with a mixture of anti-CDV antibodies/CDV+ve serum along with Protein A/G AuNP. Unfortunately, limited evidence of colouration associated with labelling was observed. Different concentrations of antigen were assessed but only subtle colouration was ever observed. This data is similar to that observed using the ELISA assay. As the surface material used for the lateral flow (nitrocellulose) is similar to that utilised in the dot blot an experiment where the two processes were combined was undertaken. Unfortunately, even in this instance, no signal was observed.

At this point, further work is needed to develop the assay prior to validation. Unfortunately, this was not possible within the time frame of the two MSc students **GL** and **CD**

Planned activity 3a: ELISA kits will be produced by the University of Kent and validated using samples held in the UK.

Planned activity 3b: ELISA kits will be distributed to international partners in Indonesia, Malaysia and Nepal, for field trials and further validation.

Output 3: Validation of the ELISA assay.

Indicator 3: The ELISA will have been trialled on archived samples, in the UK and abroad.

Expected completion date: 31/12/25

Actual completion date: Not yet completed, delays are anticipated.

Outcomes to date: Due to technical challenges and the associated delay in completing activity 2, work on activities 3a and 3b has not yet started.

Planned activity 4: collation of results and writing of reports and the paper for submission.

Output 4: Results will be shared with the conservation community

Indicator 4: Reporting to donors, publication in an open-access, peer reviewed journal and relevant media coverage.

Expected completion dates: Final report to donors 31/01/26

Submission of paper for peer review 30/09/26

Actual completion dates: Not yet completed, delays are anticipated.

Outcomes to date: MSc students GL and CD have prepared a detailed technical report on their work to date on activities 1 and 2. This has been reviewed by DB and submitted to Wildlife Vets International.

JB has prepared a mid-year report for submission to Wild Cats Conservation alliance.

Budget and spending

Budget spent to date:

Laboratory Materials: £2,064

Bench hire: £1,794

Management, report writing: £740

Total: £4,598