WILDCATS CONSERVATION ALLIANCE

Developing tools to investigate CDV in wild tigers

Project Update – June 2026

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 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.

Olivia Walters

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

Initial testing of the assay will take place in the UK, using **MGs** archived samples held at the University of Glasgow.

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 previous progress:

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 was 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.

In September 2026 WildCats agreed to a no-cost extension to facilitate further development of the assay.

Details of planned activities during no-cost extension:

Oct 2025 – Jan 2026	Refining the dot blot assay and replacing the current fluorescent marker with a visible marker. And, collation of archived serum samples from UK zoos, in preparation for validation of the assay.
	Completion of activity 2.
Feb 2026	Testing of collated archived serum samples at the University of Glasgow (using a serum neutralisation assay), and the University of Kent (using the novel dot blot assay).
	Completion of activity 3a.
Mar 2026 – Apr 2026	Any additional refinements of the dot blot assay, based on experiences during the initial round of testing. Including repeated rounds of testing, as necessary.
May 2026	Initial field trials. Distribution of dot blot assays to Nepal and testing on archived tiger and leopard samples held there.
Jun 2026	Review of the testing in Nepal, including ease of distribution, ease of testing and results.
Jul 2026	Any modifications based on the initial field trials in Nepal.
Aug 2026 – Sep 2026	Second round of field trials. Distribution of dot blot assays to all other international partners.
	Completion of activity 3b.
Oct 2026 – Nov 2026	Preparation and submission of manuscript to a peer reviewed journal.
Nov 2026	Final report to Wild Cats Conservation Alliance.
	Completion of activity 4.

Summary of progress to date

Activity 2 has been completed.

Assay development and refinement

The fluorescent marked antibody has been successfully replaced by protein A conjugated with horseradish peroxidase. This gives a blue colour while the reaction is progressing. After 15 minutes sulfuric acid is added which stops the reaction and converts the colour from blue to yellow (see table 1). Quantitative absorbance readings can then be taken using spectrophotometer, or plate reader.

Table 1. Shows the results of the newly developed assay using three control solutions, CalA, CalB and CalC, which were supplied with a commercially developed assay. The last three columns on the left show the results using various dilutions of the positive control solution CalC. The second row shows the appearance of the tubes immediately before the Sulfuric acid stop solution is added. The third row shows the appearance after the stop solution is added. This would be the final appearance of the assay. The final row shows the quantitative result which is achieved using a spectrophotometer.

	CalA Negative control	**CalB Cut off control**	**CalC Positive control**	**CalC Positive control 1:1 dilution**	**CalC Positive control 1:4 dilution**	**CalC Positive control 1: 9 dilution**
Protein A 1:20000 + TMB						
Protein A 1:20000 + Stop						
Measured absorbance at 450 nm	0.0439	0.6134	2.7571	1.1091	0.5638	0.2963

The results from the various dilutions or concentrations of the CalC positive control solution were plotted and found to show a linear relationship. This is encouraging and suggests it may be possible to determine a titre range based on the absorbance values.

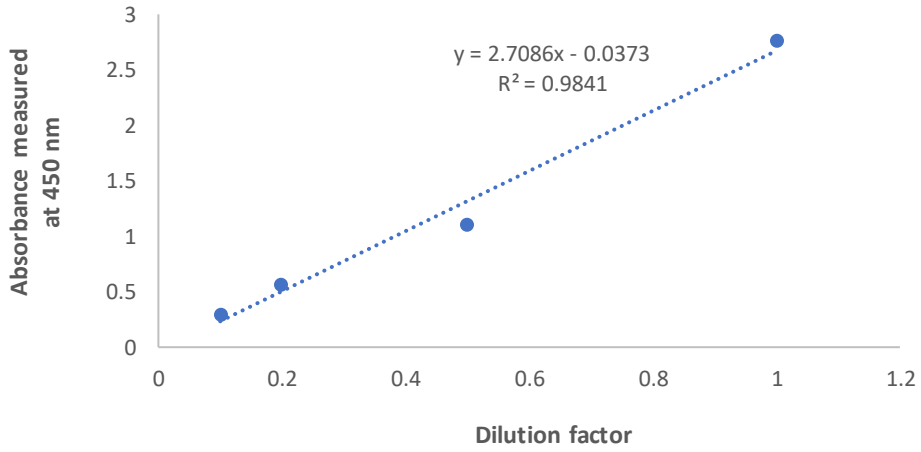

Figure 1. The relationship between the concentration of control solution CalC and final absorbance readings.

At the same time the stability of various components of the assay was investigated to determine storage requirements, a key factor for field use where access to refrigeration may be unreliable. We prepared cellulose dots using the H4 CDV antigen and compared the result with CalA negative control and CalC positive control on the freshy prepared dots, dots stored at room temp for 2 months and dots stored at -20^{0}C for 2 months and the results were comparable.

Table 2. Comparison of results using freshly prepared antigen coted cellulose dots, dots which had been prepared and then stored at-20^{0}C for 2 months and dotes which had been stored at room temp for 2 months.

	Freshly prepared Dot		**Dot stored at -20 ^{0}C for 2 months**		**Dot stored at RT for 2 months**	
	CalA Negative control	CalC Positive control	CalA Negative control	CalC Positive control	CalA Negative control	CalC Positive control
Protein A 1:20000 + TMB						
Protein A 1:20000 + Stop						

We also critically evaluated the cost of all the components of the assay as a key goal is to develop something which is affordable. Casein, which was used in blocking, was identified as the most expensive component, accounting for over 50% of the reagent costs. We investigated replacing this with skimmed milk powder, and this yielded comparable results, enabling us to dramatically reduce the material costs of the assay.

<u>Collation of archived samples for initial validation.</u>

When it came to collating archived samples for initial validation, rather than using archived UK zoo samples we elected to use field samples held by **MG** in the **University of Glasgow's** archives (table 3). This had the advantage that all the samples had already been tested using a virus neutralisation assay, so titres were already available. The archives included large numbers of canine samples, that could be used to estimate the sensitivity and specificity of the assay, as well as samples from Amur leopards and tigers that could be used to initiate the process of validating it for big cats. *(We planned to use canine samples for sensitivity and specificity calculations as the number of samples required for this, far exceeded the number of felid samples available, we estimated we would need a minimum of 100 positives and 100 negatives. Expanding the sample set to include samples from UK Zoos was unlikely to help with this, as the majority of UK collections have not been routinely vaccinated against CDV, therefore while we might be able to source additional samples this way they would likely all be negative, which would not help us in determining sensitivity and specificity).*

The fact that we had known titres for all these samples enabled us to select samples with a representative range of titres. This will help in determining a cut-off value for the colorimetric assay and enable us to determine the minimum titre that will be detected. It will also allow us to investigate whether we could have multiple categories for positive results *(i.e. positive – low titre, positive – moderate titre and positive high titre)* which could provide further information to clinicians on the ground.

Table 3. Archived samples requested from the University of Glasgow and a summary of the associated titres.

	Domestic dog (*Canis familiaris*)	**Amur leopard** (*P. pardus orientalis*) & **Amur tiger** (*P. tigris altaica*) combined
Total No. of samples	233	17
No. negative	115	8
No. positive	118	9
No. low positives (8 ≥ titre < 32)	58	1
No. moderate positives (32 ≥ titre < 240)	43	7
No. high positives (240 ≥ titre)	17	1

Unfortunately, there was a significant delay in getting the samples delivered from the University of Glasgow to the University of Kent, which delayed the start of **Activity 3a**. An update to this effect was provided to WildCats in May.

Ongoing work – activities 3a and 3b

Activity 3a was delayed by the late arrival of samples at the University of Kent. These samples have now arrived and work has begun. However, some early issues have been identified; most notably high background, which makes the signal from the assay harder to interpret.

Work to address this, i.e. the first stage of **Activity 3b** is currently ongoing. Until a solution has been identified the next stages, i.e. distribution to Nepal and other range states cannot commence.

In addition to this **JB**, who was going to lead testing in Nepal has now returned to the UK for 3 months and is now on medical leave until early August.

However, if a solution to the background problem can be identified before she returns to Nepal in September, then she can take the test kits with her and test the samples then. Kits to other countries can be distributed at the same time or earlier, meaning it may still be possible to complete the project broadly on schedule, providing a solution to the current problems can be found.