

Final report CoVetLab joint research

Maximum 4 pages;

Title Detection and differentiation of flavivirus infection for surveillance and diagnostic preparedness	
Participating partners: SVA APHA ANSES SSI WBVR NVI (external partner)	
Project leader: SVA	
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Proposal Summary (no more than 100 words) Vector-borne flaviviruses are posing a threat to both animal and human health. In Europe, there are three orthoflaviviruses (Usutu virus, USUV; West Nile virus, WNV; tick-borne encephalitis virus, TBEV) circulating that cause potentially fatal encephalitis in both humans and horses. However, clinical diagnoses as well as epidemiological studies are hampered by serological cross-reactions. This project will review what is currently known about seroprevalence in animals in Europe, and assess the methods available at the partner institutes to detect and differentiate antibodies against orthoflaviviruses. The impact of the project includes improved diagnostic expertise, which will facilitate rapid specific diagnosis of these diseases.	
Date started:	November 1, 2024
Length of project	14 months
End of project:	December 31, 2025

Project Team		
Names	Institute	Role in project
Gaëlle Gonzalez, Camille Migne, Teheipura Helle, Mathilde Gonard	ANSES	Participated in scientific discussions, contributed to study design of literature review and arranging the workshop
Johanna Lindahl, Tobias Lilja	SVA	PI, project conceptualization, grant preparation, data generation and analysis, final report writing
Melle Holwerda, Wim van der Poel, Frank Harders	WBVR	Participated in scientific discussions, data generation and analysis, contributed to study design of literature review
Ann Sofie Olesen, Louise Lohse, Thomas Bruun Rasmussen, Laura Vebæk Gelskov	SSI	Participated in scientific discussions, contributed to study design of literature review and screening of abstracts, coordination of project tasks, conducted serological analyses
Dr Karen Mansfield, Insiyah Parekh	APHA	Co-PI: project conceptualization, grant preparation, data generation and analysis, final report writing
Bieneke Bron	NVI	Participated in scientific discussions, contributed to study design of literature review and screening of abstracts

Research Questions		
1. What is the optimal pipeline for antibody testing of orthoflaviviral infections in animals in Europe for both research purposes as well as clinical diagnostics? 2. What is the current knowledge about the seroprevalence of flaviviral infections in grazing animals (horses, sheep, goats and cattle)?		
Key Objectives:		
1. To generate preliminary data on orthoflavivirus infections in Europe 2. Strengthen the capability of each participating laboratory to ensure that the correct (listed) agent is identified during an outbreak or incursion, supporting detection and control. The improved diagnostic pipelines developed will also enable larger serological studies. 3. Provide a baseline from which a future collaborative project proposal can be developed to conduct seroprevalence screening for a range of orthoflavivirus infections in mammalian species in European countries.		
Brief workplan, including key milestones		
The project started with comparing the testing pipelines of participating institutes. In continuation it was discussed which animal species we had and planned to study under the scope of the project. The project also arranged a workshop at the European Reference Laboratory (EURL) at ANSES with participants from all partner institutes. A protocol for a systematic literature review (SLR) was developed and submitted to Open Science Framework. All abstracts were screened.		
Milestone No	Milestone title	Month
1	Pipelines described	3
2	Workshop at ANSES	7
3	SLR protocol submitted	9
4	Abstracts screened	12

Planned output.(<i>deliverables, to include possible publications and IP issues</i>)		
A SLR protocol has been submitted. The plan is a published SLR of the seroprevalence of orthoflaviviruses within the European mammalian population, which is underway		
Deliverable No	Deliverable title	Month
1	Workshop	7
2	SLR protocol submitted	9
3	Abstracts screened	12

Results

The discussions on pipelines for orthoflavivirus serology revealed that most partner institutes used the ID Screen Flavivirus Competition ELISA for the initial screening of serum samples, and all partner institutes relied on the EU Reference laboratory (ANSES) to confirm results using virus neutralisation tests (VNTs). Some partners additionally used other ELISAs, such as the INgezim West Nile COMPAC ELISA or the Progen Immunozytm FSME (TBE) IgG All Species ELISA, for follow-up analyses.

This highlights a strong dependence on the EURL for confirmation of ELISA-positive samples and for discrimination between serological responses to different orthoflaviviruses.

The workshop at ANSES was highly rewarding. Many challenges related to orthoflavivirus serology were discussed, and the span of ongoing research across the different partner institutes was highlighted.

Screening of abstracts revealed 296 publications which reported having performed orthoflavivirus serology in animals in Europe. Data extraction from 256 downloaded publications are still pending.

Results from serological screenings showed that: Screening of wild boars in Sweden showed a seroprevalence of 35% (70/200) in the ID Screen Flavivirus Competition ELISA.

A cross-sectional screening of 400 horses in Sweden revealed that 23% (92/400) were positive in the ID Screen Flavivirus Competition ELISA.

In Denmark, there was no access to serum test panels from mammals during the initial part of the project period. However, orthoflavivirus serum panels, provided by ANSES, were tested at SSI using both the ID Screen Flavivirus Competition ELISA and the INgezim West Nile COMPAC ELISA. Results from this analysis revealed that the INgezim West Nile COMPAC ELISA appeared to be more specific for the Japanese encephalitis serocomplex (e.g. JEV, WNV and USUV) compared to antibodies directed against other orthoflaviviruses, such as TBEV. This finding was later confirmed, outside the scope of this project, using a panel of serum samples from horses in Denmark ($n = 49$). Using both the ID Screen Flavivirus Competition ELISA and the INgezim West Nile COMPAC ELISA at SSI, along with WNV, USUV and TBEV VNTs at ANSES, on the same panel of horse serum samples, it was demonstrated that the INgezim West Nile COMPAC ELISA was more specific towards WNV and USUV compared to the ID Screen Flavivirus Competition ELISA.

A panel of sera from grazing animals in the UK was assessed ($n=34$ samples) using the Progen Immunozytm FSME (TBE) IgG All species ELISA, for determination of IgG- antibodies against TBEV. Samples originated from deer ($n=1$), cattle ($n=20$) and horses ($n=13$). The results demonstrate the presence of anti-TBEV antibodies in horses and cattle (**Table 1**). This included horse sera that were previously confirmed to be negative or doubtful for anti-WNV antibodies using the IDVet ELISAs. However, this is based on ELISA data only, hence for confirmation of results and to make firm conclusions, further testing by virus neutralisation assay (e.g., PRNT) would be required.

Table 1: Anti-TBEV antibodies in grazing animals in the UK (determined by ELISA). Percentages shown in parentheses.

Sample	Sample Type	Prior Testing	Total Number (n)	TBEV-ELISA Results		
				Positive	Borderline	Negative
Horse diagnostic submissions	Serum	WNV ELISA*	3	2 (66.6)	0 (0)	1 (33.3)

		(Negative IgM, doubtful IgG)				
Horse diagnostic submissions	Serum	WNV ELISA* (Negative IgM, negative IgG)	10	2 (20)	5 (50)	3 (30)
Cattle (research)	Blood	Research (molecular)**	18	1 (5.6)	4 (22.2)	13 (72.2)
Cattle (research)	Serum	Research (molecular)**	2	2 (100)	0 (0)	0 (0)
Deer (research)	Blood	Research (molecular)**	1	0 (0)	0 (0)	1 (100)
<i>*Commercially available WNV ELISAs: (IDScreen West Nile IgM Capture and IDScreen Competition Multi-Species</i> <i>** Molecular screening for Babesia spp. and Anaplasma phagocytophilum.</i>						
Problems experienced						
Cross-reactivity remains a major challenge in orthoflavivirus serology. A multiplex serological assay for orthoflaviviruses could not be finalized, and milestones and deliverables were adjusted accordingly during the course of the project. However, work is still on-going. Efforts are continuing at both ANSES and SVA in collaboration with Uppsala University, to develop a multiplex suspension immunoassay, which may prove sufficiently specific in the future.						
Value of cooperation for CoVetLab institutes						
Orthoflavivirus serology remains complex, with inherent issues related to cross-neutralisation in ELISAs. However, until improved methods are validated and available, the present testing pipeline is the best. This requires the strong collaboration with the EU Reference laboratory (ANSES), which was strengthened during this project.						
Any other information						
Evaluation						
Deliverables met?	It was not possible to finalize an alternative discriminatory test during this project, but there are two ongoing methods being tested.					
Start of project						
Cooperation	Cooperation was strengthened during this project, and there are plans to potentially submit future applications by the consortium					
Advice towards new calls / new projects	Future projects on flaviviruses should also include the less known viruses. It would be good to plan for two workshops in real life and rely less on digital meetings.					