

Final report CoVetLab joint research

Maximum 4 pages;

Title (no more than 100 characters) Optimizing sampling protocols for Aujeszky's disease diagnostics		
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Project Summary (max 250 words) Infections with Aujeszky's disease virus (ADV) in <i>Suidae</i> are listed in the Animal Health Law. The virus is present in wild boar populations in several countries in the EU. ADV in wild boar populations poses a constant threat to the ADV-free status in domestic pigs of EU countries. Recent ADV outbreaks in domestic pigs (e.g. France) make it essential to optimize tools for sampling of pigs and conditions for transport and storage of samples for ADV laboratory diagnosis in case of AD suspect cases and outbreaks. In this project, we aimed to optimize sampling protocols for laboratory diagnosis of ADV in pigs in the collaborating countries. We compared the in-use diagnostic protocols of the collaborating partners. We also designed in vitro experiments to assess various sampling tools (e.g. various swabs), transport media and storing conditions. This part of study was carried out using several ADV isolates due to limited availability of clinical materials. Several virus strains were shared among partners to assess sampling tools, to spike media and to evaluate virus infectivity and stability during storage. Subsequently, clinical samples obtained from experimentally infected animals, from an experimental study carried out at ANSES, were used to further evaluate the sampling tools and transport media selected from the <i>in vitro</i> experiments. Finally, the gathered knowledge on sampling tools, transport media and storage conditions in this project could be the basis for harmonization of ADV sampling and diagnostics across partner institutes.		
Length of project in months	Months: 18	Start Date: 1st Oct 2024
Workplan & Timelines (GANTT chart): see proposal		
Costs		
Proposed total budget (k€): see proposal		
Research Questions: see above		
Key Objectives: see above		
Brief workplan, including key milestones: see proposal and below		

Planned output.(<i>deliverables, to include possible publications and IP issues</i>)
Task 1: Review current ADV sampling protocols • Literature review: sample types, swab types, media and stability of ADV in the environment • Each partner: current diagnostic assays, protocols and available isolates Task 2: <i>In vitro</i> study • Physical “study design meeting” at SSI: discussion on information from task 1 and the protocol for the <i>in vitro</i> study • ANSES: production of two viral stocks and dispatch to partners for the <i>in vitro</i> study • APHA, SSI + WBVR: performance of the <i>in vitro</i> experimental study Task 3: Experimental infection • ANSES: <i>in vivo</i> study with 2 different ADV strains: evaluation of clinical signs, sample collection and analysis. • WBVR: Short-term visit to ANSES Task 4: Laboratory analyses of <i>in vivo</i> material • ANSES: Sample analysis and selection of samples to be analysed similar to an ILCT at the other partners • APHA, SSI + WBVR: PCR analysis of selected samples Task 5: Writing report & scientific paper • Physical “final meeting” at WBVR: discuss results and final report as well as publication and future plans

Results (maximum 1,5 page)
Task 1: Review current ADV sampling protocols Results discussed during the physical “study design meeting”. Task 2: <i>In vitro</i> study Although it was originally planned that all partners would carry out the <i>in vitro</i> study, it was decided not to do this at ANSES because of their workload associated with the <i>in vivo</i> study. The <i>in vitro</i> experiments were conducted at APHA, SSI and WBVR using a local ADV isolate and two reference strains (Bartha and NIA3, provided by ANSES). Each strain was applied to cotton and flocked swabs, both with and without transport media. The swabs were then stored at three different temperatures (4 °C, room temperature, and 37 °C) for 0, 24, 48, 72, and 96 hours. Sample preparation, DNA extraction and qPCR testing were carried out according to the standard protocols of each laboratory. <u>The preliminary results (see attachment for poster which was presented at ESPHM 2026)</u> demonstrate that swab type and the type of medium do not influence the sensitivity of ADV detection by gB qPCR, when samples are stored up to 96 hours at 4°C, room temperature, and 37°C. However, the presence of transport medium seems to influence the results, with dry swabs showing higher Ct-values compared to swabs stored with transport medium. Statistical analysis is planned, as well as further work to determine how these conditions might influence virus viability (see below) and to validate these data using clinical samples from experimentally infected animals (see tasks 3+4).

Virus viability/infectivity has been investigated by all partners, according to the standard protocols of each laboratory through virus isolation, CPE observation and immunostaining. Preliminary results discussed during the final meeting: cold, wet storage and swabs with no wooden handle seem to work best for virus isolation. Further (statistical) analysis is planned.

Task 3: Experimental infection

An experimental infection (approved by the ethics committee and authorized by the French Ministry of Research) was performed at ANSES. The objectives of the study were to:

- Compare the diagnostic performance of different sample collection methods
- Test alternative sampling approaches for diagnosis (e.g. oral fluid, nasal wipes, air sampling)
- Collect positive tissues for the reference activities of the WOAHL RL and NRLs

Next to the clinical observation of the pigs, various samples were collected at different timepoints: blood samples, nasal and oropharyngeal swabs (using two swab types), tonsil brushings, individual oral fluids, nasal wipes and air samples. Also, necropsies were performed and various tissue samples were collected for PCR analysis and for histological analysis at WBVR. Until now, almost all samples are analysed at ANSES using PCR diagnostics and the preliminary results were discussed during the final meeting. Histological evaluation, as well as statistical analysis of the results are planned.

Preliminary results discussed during the final meeting: individual oral fluids were shown not suitable for serological analysis using ADV gB ELISA. In addition, the PCR Ct-values differed among sample types: tonsil brushing seems to be more sensitive than nasal and oropharyngeal swabs, since the tonsillar brush samples were positive until the end of the study (also in asymptomatic infected pigs). Tonsils seem to be the “best organs” for ADV detection providing the best sensitivity.

Task 4: Laboratory analyses of *in vivo* material

Based on the results of the *in vivo* study, ANSES selected 60 samples, including nasal and oropharyngeal swabs (both cotton and flocked) and tonsillar brushes. These were shipped to APHA, SSI and WBVR to be analysed similar to an inter-laboratory comparison test (ILCT). The results of all partners were compared and discussed during the final meeting, at which the preliminary conclusion was that “all labs succeeded”.

Task 5: Writing report & scientific paper

Next to this report, the (preliminary) results of the *in vitro* study were presented as a poster at ESPHM 2026. Submission of an abstract to the Epizone conference, and two scientific papers (*in vitro* and *in vivo* studies) to peer-reviewed journals are in progress but could not be finalised within the timeline of the project.

Problems experienced	
The <i>in vitro</i> study was rather complicated (different combinations and conditions), requiring longer preparation time and some communication difficulties leading to delays and in some cases also to repeated testing. The budget was (as expected) not sufficient to cover additional costs incurred, for instance: the time and costs for publication, and for the statistical analysis of the data by WBVR epidemiologist. Additionally, pathology was not included in the original plan of the <i>in vivo</i> study, however, we included this important part to maximize the value of the animal study, resulting in additional costs.	
Value of cooperation for club 5 institutes	
• Collaboration in the absence of an EURL • Opportunity to visit other institutes • Raise awareness of ADV, review of existing protocols, and alert both research institutes and field professionals (including authorities and veterinarians). Therefore: dissemination of results is considered highly important: ○ To be prepared for such “not so common” notifiable diseases and suspicions ○ To be aware that not every swab/sample type is adequate for ADV laboratory diagnosis (N.B. Extrapolation/additional research needed for other pathogens?)	
Any other information	
<u>We were happy with:</u> • MTA's + shipment of strains and samples • Meeting frequency • Teams-page <u>More attention & (future) budget needed for:</u> • All partners provided additional funding for this project to varying degrees, as the initial budget was insufficient. • Additional budget needed for: ○ Involvement of pathology & epidemiology teams at WBVR ○ Time and costs for publication • Dissemination of results is considered very important, but will only happen after the finalization of the project. <u>Ideas:</u> • Travel funding to maintain collaboration in the absence of an EURL and to disseminate results to other NRLs.	
Evaluation	
Deliverables met?	Not completely within the timeline of the project due to additional tasks undertaken, but remaining deliverables will be met and disseminated through publications.
Start of project	See above. Additionally: “in-person” study design meeting was very useful, especially to get to know each other.
Cooperation	See above
Advice towards new calls / new projects	See above