

## Final report CoVetLab joint research

Maximum 4 pages;

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| Title (no more than 100 characters)<br><b>Interpreting and optimising bulk milk testing for BVD and IBR considering numerical test outcomes</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                |
| Participating partners: ANSES, APHA, SSI, SVA, UCPH, WBVR                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                                |
| Project leader                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                |
| Name: Arianna Comin                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                |
| Email address and telephone number: <a href="mailto:arianna.comin@sva.se">arianna.comin@sva.se</a> +46 (0) 18 674 079                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                |
| Summary (no more than 100 words)<br>Many diagnostic assays convert numerical results into positive/negative outcomes, losing valuable detail. This project developed Bayesian latent class models (BLCMs) to improve interpretation of serological test data by incorporating numerical outcomes. Building on a previous CoVetLab project on infectious bovine rhinotracheitis (IBR) and bovine viral diarrhoea (BVD), we extended the approach to bulk-milk testing to enhance diagnostic accuracy and comparability across laboratories. The probability of a herd being truly seropositive for BVD/IBR from bulk-milk test results was determined by comparing serological test performance across laboratories. We developed tools to optimise country-specific diagnostics and a dashboard to interpret infection probabilities based on numerical test outcomes. |                                |
| Date started:                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | 1 <sup>st</sup> October 2024   |
| Length of project                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | 14 months                      |
| End of project:                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 30 <sup>th</sup> November 2025 |

| Project Team             |           |                                                                 |
|--------------------------|-----------|-----------------------------------------------------------------|
| Names                    | Institute | Role in project                                                 |
| Arianna Comin            | SVA       | Project coordination, method development, result interpretation |
| Viktor Ahlberg           | SVA       | Sample management, method development, result interpretation    |
| Matt Denwood             | UCPH      | Method development, result interpretation                       |
| Bertel Strandbygaard     | SSI       | Lab analysis, result interpretation                             |
| Louise Lohse             | SSI       | Lab analysis, result interpretation                             |
| Mark Arnold              | APHA      | Method development, result interpretation                       |
| Adriana Diaz Alvarado    | APHA      | Method development, result interpretation                       |
| Amin Asfor               | APHA      | Sample management, lab analysis, result interpretation          |
| Aldo Dekker              | WBVR      | Lab analysis, result interpretation                             |
| Eduardo de Freitas Costa | WBVR      | Method development, result interpretation                       |
| Stephen Valas            | ANSES     | Sample management, lab analysis, result interpretation          |
| Guy Kouokam              | ANSES     | Sample management, lab analysis, result interpretation          |

| Research Questions                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <ol style="list-style-type: none"><li>1. What additional information from numerical test outcomes can be used with serological tests?</li><li>2. What is the diagnostic performance of tests currently used by partner institutes to detect the presence of IBR and BVD in bulk milk?</li><li>3. What is the probability of a herd being truly seropositive for BVD or IBR given a certain bulk milk test result and expected herd-level prevalence?</li></ol>         |
| Key Objectives:                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| <ol style="list-style-type: none"><li>1. Develop a BLCM method to analyse numerical test outcomes from multiple diagnostic tests</li><li>2. Compare the test performance of routine bulk milk diagnostics for BVD and IBR</li><li>3. Develop tools to optimise test performance for country-specific requirements</li><li>4. Develop a dashboard for test result interpretation that provides the probability of infection given the numerical test outcome.</li></ol> |

| Brief workplan, including key milestones                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                     |       |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|-------|
| <p><u>Task 1: Collection of background information</u></p> <p>ANSES, APHA, SSI, SVA and WBVR compiled a detailed description of the diagnostic tests used in their laboratories to detect the presence of IBR and BVD in bulk milk, including characteristics such as specific lab procedures, cut-off values, etc.</p> <p><u>Task 2: Development of statistical approach based on BLCM</u></p> <p>APHA and UCPH, together with SVA and WBVR, explored statistical approaches for inter-laboratory diagnostic test evaluation using numerical test outcomes. Existing work on BLCMs accounting for several tests simultaneously evaluated in multiple populations with varying disease prevalence were expanded to use additional information from numerical test results.</p> <p><u>Task 3: Sample management</u></p> <p>The project involved collecting and analysing 500 samples per disease, which represented a good compromise between statistical considerations and budget constraints. ANSES, APHA and SVA collected bulk milk samples from ongoing surveillance activities in France (FR), United Kingdom (UK) and Sweden (SE). Each selected sample was split in five parts: one for each analysing laboratory. In this way, the same set of samples were analysed by all laboratories.</p> <p><u>Task 4: Laboratory analyses</u></p> <p>The bulk milk samples collected in Task 3 were distributed between ANSES, APHA, SSI, SVA and WBVR, and analysed using diagnostic assays described in Task 1. Each laboratory used their own tests and lab procedures, as the goal of the project was to evaluate what is currently in use in each laboratory.</p> <p><u>Task 5: Estimation of test accuracy and result interpretation</u></p> <p>The results of the tests performed in the different laboratories under Task 4 was collated in a shared private repository to be jointly analysed using the algorithms and tool developed in Task 2. The final output was an estimate of the performance of each test and the probability of a herd being truly seropositive given a certain bulk milk test result and expected herd-level prevalence.</p> <p><u>Task 6: Development of a dashboard for test optimisation and interpretation</u></p> <p>The output of Task 5 can be visualised in a dashboard where the user can change the input values and have a dynamic evaluation of test performances. The dashboard can also aid the interpretation of the test result based on country-specific herd-level prevalences and without relying on a defined cut-off level.</p> |                                     |       |
| Milestone No                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | Milestone title                     | Month |
| 1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | Kick-off meeting                    | 2     |
| 2                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | Samples shipped to all labs         | 8     |
| 3                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | Lab analyses concluded              | 10    |
| 4                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | Final meeting with joint discussion | 12    |

| Planned output. ( deliverables, to include possible publications and IP issues) |                        |                   |
|---------------------------------------------------------------------------------|------------------------|-------------------|
|                                                                                 |                        |                   |
| Deliverable No                                                                  | Deliverable title      | Month             |
| 1                                                                               | Dashboard              | 15                |
| 2                                                                               | Final report           | 15                |
| 3                                                                               | Scientific publication | after project end |

**Results ( maximum 1,5 page)**

For BVD, 500 bulk milk samples from FR (n=150), SE (n=100) and UK (n=250) were analysed with 5 different commercial ELISA kits (some kits were the same across laboratories):

| Commercial kit names                         | Test protocol names (including lab running it) |
|----------------------------------------------|------------------------------------------------|
| IDVet ID Screen BVD p80 Antibody Competition | IDVetL_ANSES, IDVetS_ANSES, IDVetL_SSI         |
| IDEXX BVDV p80Ab                             | IDEXX_ANSES                                    |
| SVANOVIR BVDV-Ab ELISA – confirmatory        | Svanova_APHA                                   |
| SVANOVIR BVDV-Ab ELISA – screening           | Svanova_SVA                                    |
| PrioCHECK Bovine BVDV Ab Plate Kit           | PrioCheck_WBVR                                 |

For IBR, 500 bulk milk samples from FR (n=100), SE (n=100) and UK (n=300) were analysed with 5 different commercial ELISA kits (some kits were the same across laboratories):

| Commercial kit names                | Test protocol names (including lab running it) |
|-------------------------------------|------------------------------------------------|
| IDEXX IBR Tank Milk                 | IDEXX_tank_ANSES                               |
| IDEXX IBR Pool                      | IDEXX_pool ANSES, IDEXX_pool APHA              |
| IDVet ID Screen IBR Milk Indirect   | IDVetS_ANSES, IDVetS_SVA, IDVetL_SSI,          |
| PrioCHECK BHV-1 gB ELISA            | PrioCheckgB_WBVR                               |
| IDEXX BHV-1 gE XM Antibody Test Kit | IDEXXgE_WBVR                                   |

The first modelling approach consisted of applying mixture models to each test separately (i.e., univariate) using the best-fitting distribution (i.e. normal, log-normal or gamma) for the numerical test outcomes of positive (P) and negative (N) samples. The second modelling approach consisted of six pairwise comparisons against one test (e.g. IDVetL\_ANSES), including an additional correlation term ( $\rho$ ) between tests. Lastly, the probability of infection for the numerical outcome of a given test and prevalence combinations was calculated applying Bayes' theorem using likelihoods from the log-normal probability density function.

Here is an example of the estimates of diagnostic performance of two test protocols for BVD using the two modelling approaches. The table shows the posterior means and 95% credible intervals for sensitivity (Se), Specificity (Sp) and prevalence (prev) for ANSES\_IDVetL and ANSES\_IDEXX:

| Parameter | ANSES_IDVetL [1]<br>Univariate log-normal | ANSES_IDEXX [2]<br>Univariate log-normal | Correlated ANSES IDVetL[1]<br>& ANSES_IDEXX [2]<br>Bivariate log-normal |
|-----------|-------------------------------------------|------------------------------------------|-------------------------------------------------------------------------|
| FR prev   | 0.896 (0.847-0.943)                       | 0.884 (0.825-0.941)                      | 0.895 (0.846-0.942)                                                     |
| SE prev   | 0.0001(0.0000-0.0004)                     | 0.0001(0.0000-0.0004)                    | 0.0001(0.0000-0.0004)                                                   |
| UK prev   | 0.955 (0.928-0.980)                       | 0.907 (0.862-0.947)                      | 0.942 (0.910-0.970)                                                     |
| Se[1]     | 0.990 (0.984-0.995)                       |                                          | 0.993 (0.988-0.997)                                                     |
| Se[2]     |                                           | 0.952 (0.934-0.969)                      | 0.944 (0.927-0.961)                                                     |
| Sp[1]     | 1.00 (1.00-1.00)                          |                                          | 1.00 (1.00-1.00)                                                        |
| Sp[2]     |                                           | 0.928 (0.891-0.964)                      | 0.951(0.923-0.976)                                                      |
| $\rho_P$  |                                           |                                          | 0.832 (0.802-0.864)                                                     |
| $\rho_N$  |                                           |                                          | -0.135 (-0.315-0.041)                                                   |

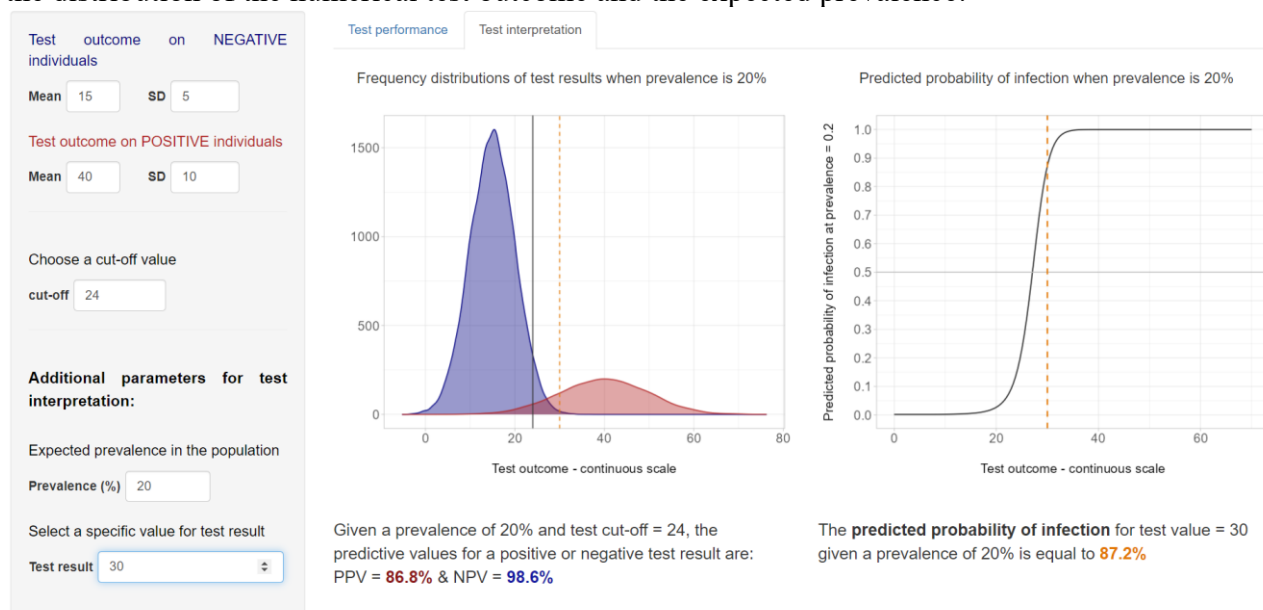
Overall, the univariate and bivariate prevalence estimates were in good agreement, and almost all tests showed high sensitivity and specificity. The estimated sensitivity of PrioCheck\_WBVR was the lowest across all tests and simulations (<84%), with a markedly lower value in the bivariate model (65.8%) compared with the univariate model (83%). As expected, the BVD IDVet tests showed positive correlations, both between tests from the same laboratory (ANSES) and across laboratories (ANSES vs. SSI), as well as between the short (S) and long (L) protocol variants of the same assay. Notably, these correlations were stronger among positive results than negative ones. In contrast, for the other three BVD tests their correlations with the IDVetL\_ANSES test were negative among positive results, and the correlations among positives were weaker than among negatives.

Here is an example of the probability of BVD infection based on some possible numerical outcomes of IDVetL\_ANSES and different expected herd-level prevalence levels:

| Prevalence \ Numerical test outcome of IDVetL_ANSES* |      |      |      |      |         |         |         |         |        |        |        |
|------------------------------------------------------|------|------|------|------|---------|---------|---------|---------|--------|--------|--------|
|                                                      | 12   | 60   | 63   | 65   | 67      | 70      | 75      | 80      | 85     | 90     | 100    |
| 1%                                                   | 100% | 100% | 100% | 100% | 99.998% | 99.749% | 36.759% | 0.458%  | 0.015% | 0.002% | 0.000% |
| 5%                                                   | 100% | 100% | 100% | 100% | 100%    | 99.952% | 75.177% | 2.343%  | 0.078% | 0.008% | 0.002% |
| 20%                                                  | 100% | 100% | 100% | 100% | 100%    | 99.990% | 93.500% | 10.230% | 0.368% | 0.039% | 0.008% |
| 50%                                                  | 100% | 100% | 100% | 100% | 100%    | 99.997% | 98.292% | 31.310% | 1.456% | 0.157% | 0.031% |
| 75%                                                  | 100% | 100% | 100% | 100% | 100%    | 99.999% | 99.424% | 57.761% | 4.244% | 0.468% | 0.093% |

\* cut-off for positive test result:  $\leq 65$

Here is an example of the dashboard output aiding the diagnostic interpretation of a test based on the distribution of the numerical test outcome and the expected prevalence:



## Problems experienced

The project timeline was tight due to long lead times for diagnostic sample collection, laboratory analyses and statistical modelling. Samples could only be aliquoted, labelled, and shipped to partner laboratories once collection was fully completed. For French samples, targeted sampling of positive cases also required waiting for outbreak suspicion. Additional delays arose from suspected label switching in a subset of samples, confirmed late in the project, which required reshipping and re-analysis. This incident emphasized the importance of rigorous sample handling and clear communication across laboratories.

Preliminary evaluations based on simulated data proved of limited value because the real test-result distributions differed from those assumed in the simulations. The project highlighted that BLCMs for continuous data are more complex than anticipated. In particular, the IBR results were truncated, with many samples reaching the assay's detection limit and returning the same maximum value within a plate. This made it impossible to fit a meaningful distribution for the mixture models. As a result, traditional cutoff-based BLCMs were fitted in parallel to provide laboratories with practical performance estimates and a new non-parametric modelling method was also theorised.

## Value of cooperation for CoVetLab institutes

The diagnostic evaluations would not have been possible without close collaboration among the partner institutes. Sample collection, laboratory analyses, and modelling all benefitted from pooling expertise, especially the combination of laboratory specialists with epidemiologists and statisticians.

The test performance results can now be directly applied in routine diagnostics at partner institutes. The project also strengthened a professional network involving laboratory experts in BVD and IBR as well as epidemiologists and modellers. This cooperation helped bridge the gap between laboratory and epidemiological perspectives, creating a useful platform for ongoing dialogue and future diagnostic initiatives.

#### **Any other information**

For SVA, the project was co-funded by the EUP Animal Health & Welfare SOA12 (BETO)

#### **Evaluation**

|                                         |  |
|-----------------------------------------|--|
| Deliverables met?                       |  |
| Start of project                        |  |
| Cooperation                             |  |
| Advice towards new calls / new projects |  |