**The STROBE-Vet statement checklist.**

| | *Item* | *STROBE-Vet recommendation* | *Page #* |
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| **Title and Abstract** | 1 | (a) Indicate that the study was an observational study and, if applicable, use a common study design term | The title states that this is a molecular survey on pigs (observational). The abstract specifies that this is a repeated cross-sectional observational study. p. 1 |
| | | (b) Indicate why the study was conducted, the design, the results, the limitations, and the relevance of the findings | The abstract includes the rationale, the design, the results, the limitations, and the public health relevance. p. 1 |
| **Background / rationale** | 2 | Explain the scientific background and rationale for the investigation being reported | The background section provides scientific background and detailed rationale. pp. 1–3 |
| **Objectives** | 3 | (a) State specific objectives, including any primary or secondary prespecified hypotheses or their absence | The objectives are clearly stated in the last paragraph of the Background section. p. 3 |
| | | (b) Ensure that the level of organisation[a] is clear for each objective and hypothesis | The objectives include investigating pigs of different age groups. p. 3 |
| **Study design** | 4 | Present key elements of study design early in the paper | The type of the study and key elements are reported at the beginning of the Methods section. pp. 3–4 |
| **Setting** | 5 | (a) Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | All relevant information regarding the setting, locations, and dates, including sampling |

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| | | | periods and data collection, is reported in the “Sampling and data collection” subsection of the Methods section. pp. 3–4 |
| | | (b) If applicable, include information at each level of organization | Farm-level and pig-level described. pp. 3–4 |
| **Participants** [b] | 6 | (a) Describe the eligibility criteria for the owners/managers and for the animals, at each relevant level of organization | Farms were eligible if they were intensive pig farms located in northern Italy and the farmers agreed to participate. Pigs were eligible if they were apparently healthy fattening pigs of the specified age range. p. 3 |
| | | (b) Describe the sources and methods of selection for the owners/managers and for the animals, at each relevant level of organization | Within each farm, several pens representing different stages of the fattening cycle were randomly selected, and two pigs per pen were randomly chosen by the veterinarian without preferential criteria. Farms were selected based on willingness to participate. pp. 3–4 |
| | | (c) Describe the method of follow-up | Two sampling sessions: beginning and end of fattening cycle. Distinct batches, each sampling session included pigs available at that time. pp. 3–4 |
| | | (d) For matched studies, describe matching criteria and the number of matched individuals per subject (e.g., number of controls per case) | Not applicable. |
| **Variables** | 7 | (a) Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. If applicable, give diagnostic criteria | The primary outcome was *E. bieneusi* positivity based on nested PCR targeting the ITS region, followed by sequencing |

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| | | | for genotype identification. Predictors included age category, application of all-in/all-out system, faecal soiling score, farm size, and sampling season. pp. 4–5 |
| | | (b) Describe the level of organization at which each variable was measured | Farm- and animal-level. pp. 4–5 |
| | | (c) For hypothesis-driven studies, the putative causal-structure among variables should be described (a diagram is strongly encouraged) | No formal causal diagram was developed. |
| **Data sources / measurement** | 8* | (a) For each variable of interest, give sources of data and details of methods of assessment (measurement). If applicable, describe comparability of assessment methods among groups and over time | For each variable, sources of data and details of methods were provided. The same sampling and laboratory procedures were applied consistently across farms and between the two sessions. pp. 3–5 |
| | | (b) If a questionnaire was used to collect data, describe its development, validation, and administration | Not applicable. Data were collected in a form for each farm with relevant variables, not through a questionnaire. p. 4 |
| | | (c) Describe whether or not individuals involved in data collection were blinded, when applicable | Not applicable. |
| | | (d) Describe any efforts to assess the accuracy of the data (including methods used for "data cleaning" in primary research, or methods used for validating secondary data) | Data accuracy was ensured through manual verification of PCR amplification and sequencing outputs prior to analysis. p. 5 |
| **Bias** | 9 | Describe any efforts to address potential sources of bias due to confounding, selection, or information bias | Selection bias was reduced by applying a random sampling strategy within each farm. Information bias was limited by using standardised protocols for sample and data collection, |

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| | | | processing, PCR testing, and sequencing, all performed by the same qualified personnel. pp. 3–5 |
| **Study size** | 10 | (a) Describe how the study size was arrived at for each relevant level of organization | The calculation of the minimum sample size. p. 3 |
| | | (b) Describe how non-independence of measurements was incorporated into sample-size considerations, if applicable | Not applicable. |
| | | (c) If a formal sample-size calculation was used, describe the parameters, assumptions, and methods that were used, including a justification for the effect size selected | The calculation of the minimum sample size assumed a population of 2 million pigs in northern Italy, an expected prevalence of 50%, a confidence interval of 95% and an absolute precision of 5%. A statistical tool was used. p. 3 |
| **Quantitative variables** | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why | Quantitative variables were categorised for the analysis. p. 4 |
| **Statistical methods** | 12 | (a) Describe all statistical methods for each objective, at a level of detail sufficient for a knowledgeable reader to replicate the methods. Include a description of the approaches to variable selection, control of confounding, and methods used to control for non-independence of observations | *Enterocytozoon bieneusi* positivity (binary outcome) was analysed using GLMs; variables with $p$-value < 0.1 in univariate analyses were included in multivariate models using backward selection based on AIC. p. 5 |
| | | (b) Describe the rationale for examining subgroups and interactions and the methods used | No formal analyses of subgroups or interactions were conducted beyond categorical comparisons of selected variables. p. 5 |
| | | (c) Explain how missing data were addressed | No missing data were present for variables included in the analyses. p. 5 |

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| | | (d) If applicable, describe the analytical approach to loss to follow-up, matching, complex sampling, and multiplicity of analyses | Not applicable. |
| | | (e) Describe any methods used to assess the robustness of the analyses (e.g., sensitivity analyses or quantitative bias assessment) | Model robustness was assessed using standard criteria (AIC, significance of variables). p. 5 |
| **Participants** | 13* | (a) Report the numbers of owners/managers and animals at each stage of study and at each relevant level of organization - e.g., numbers eligible, included in the study, completing follow-up, and analysed | 22 farms participated, with 440 pigs sampled across two sessions (220 per session). Within each farm, 20 pigs were randomly selected (10 at the beginning and 10 at the end of the fattening cycle). pp. 3–4 |
| | | (b) Give reasons for non-participation at each stage and at each relevant level of organization | No farms or animals were excluded after selection; all selected pigs were included in the analysis. |
| | | (c) Consider use of a flow diagram and/or a diagram of the organizational structure | Not applicable. |
| **Descriptive data on exposures and potential confounders** | 14* | (a) Give characteristics of study participants (e.g., demographic, clinical, social) and information on exposures and potential confounders by group and level of organization, if applicable | Characteristics of pigs and farms are described in "Sampling and data collection" subsection of the Methods section. pp. 3–4 |
| | | (b) Indicate number of participants with missing data for each variable of interest and at all relevant levels of organization | No missing data were found for either animals or variables. |
| | | (c) Summarize follow-up time (e.g., average and total amount), if appropriate to the study design | Not applicable. |
| **Outcome data** | 15* | (a) Report outcomes as appropriate for the study design and summarize at all relevant levels of organization | *E. bieneusi* positivity is reported at the animal and farm levels for each sampling session. pp. 5–6 |
| | | (b) For proportions and rates, report the numerator and denominator | Proportions are presented with numerators and denominators, see Table 1. |

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| | | (c) For continuous outcomes, report the number of observations and a measure of variability | Not applicable. |
| **Main results** | 16 | (a) Give unadjusted estimates and, if applicable, adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders and interactions were adjusted. Report all relevant parameters that were part of the model | The adjusted estimates derived from univariate and multivariate models are shown in Tables 1 and 2. |
| | | (b) Report category boundaries when continuous variables were categorized | See Table 1. |
| | | (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | Not applicable. |
| **Other analyses** | 17 | Report other analyses done,-such as sensitivity/robustness analysis and analysis of subgroups | Not applicable. |
| **Key results** | 18 | Summarize key results with reference to study objectives | Key results are summarised in the Discussion, highlighting prevalence estimates, risk factors, and molecular findings in relation to the study objectives. pp. 7–9 |
| **Strengths and Limitations** | 19 | Discuss strengths and limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | Strengths and limitations are discussed in the manuscript. p. 7 |
| **Interpretation** | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | A cautious overall interpretation of results is provided in the Discussion, considering study objectives, limitations, comparison with similar studies, and implications for public health. pp. 7–9 |
| **Generalizability** | 21 | Discuss the generalisability (external validity) of the study results | Generalisability is discussed in the manuscript. p. 7 |
| **~~Funding~~ Transparency** | 22 | (a) Funding- Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | This study was entirely funded by internal resources of the authors' institution (see Funding section). p. 10 |

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| | | (b) Conflicts of interest-Describe any conflicts of interest, or lack thereof, for each author | All authors declare no conflicts of interest (see Competing Interests section). p. 10 |
| | | (c) Describe the authors' roles-Provision of an authors' declaration of transparency is recommended | Authors' contributions are detailed in the manuscript (see the Authors' Contributions section). p. 10 |
| | | (d) Ethical approval- Include information on ethical approval for use of animal and human subjects | Animal sampling was approved by the Institutional Animal Care and Use Committee of Università degli Studi di Milano, Protocol number: OPBA_90_2023 (see Ethics Approval and Consent to Participate section). p. 10 |
| | | e) Quality Standards-Describe any quality standards used in the conduct of the research | Sampling and laboratory procedures followed standard protocols, including Classyfarm cleanliness scoring and validated PCR methods. pp 4–5 |

[a] Level of organization recognizes that observational studies in veterinary research often deal with repeated measures (within an animal or herd) or animals that are maintained in groups (such as pens and herds); thus, the observations are not statistically independent. This non-independence has profound implications for the design, analysis, and results of these studies.
[b] The word "participant" is used in the STROBE statement. However, for the veterinary version, it is understood that "participant" should be addressed for both the animal owner/manager and for the animals themselves.
*Give such information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.