ARRIVE Guidelines Checklist

**Subject:** BVET-D-20-00665

**Title:** *De novo* characterization of the genetic polymorphism and transcript abundance of Toll-like receptors (TLRs) in tissues of swamp buffaloes (Bubalus bubalis) from Guangxi, China

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| **ITEM** | **ESSENTIAL 10** | **CRITERION** | **SECTION** | **PARAGRAPH  (WITHIN SECTION)** |
| **1** | **Study design** | 1. The group being compared, including control group. If no control group has been used, the rationale should be stated. 2. The experimental unit (e.g. a single animal, litter, or cage of animals). | Methods | 1. This is a DNA sequences analyses – based study on healthy normal animals (but not a comparative study), so no control group was included. 2. Paragraph 1 |
| **2** | **Sample size** | 1. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. 2. Explain how the sample size was decided. Provide details of any a priori sample size calculation, if done. | Methods | 1. Paragraph 1 2. Paragraphs 4, 5, 6 |
| **3** | **Inclusion and exclusion criteria** | 1. Describe any criteria used for including or excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established a priori. If no criteria were set, state this explicitly. 2. For each experimental group, report any animals, experimental units, or data points not included in the analysis and explain why. If there were no exclusions, state so. | Methods | 1. Paragraph 1 2. No exclusions |
| **4** | **Randomisation** | 1. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. 2. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly. | N/A | N/A |
| **5** | **Blinding** | Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis). | N/A | N/A |
| **6** | **Outcome measures** | 1. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). 2. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size. | Methods | 1. Paragraphs 2, 4, 5, 6 2. N/A |
| **7** | **Statistical methods** | 1. Provide details of the statistical methods used for each analysis, including software used. 2. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met. | Methods | Paragraph 6 |
| **8** | **Experimental animals** | 1. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. 2. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures. | Methods | Paragraph 1 |
| **9** | **Experimental procedures** | 1. What was done, how it was done, and what was used. 2. When and how often. 3. Where (including detail of any acclimatisation periods). 4. Why (provide rationale for procedures). | Background | Paragraph 4 |
| Methods | Paragraphs 1-11 (Throughout) |
| **10** | **Results** | 1. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). 2. If applicable, the effect size with a confidence interval. | Results | Paragraphs 15-16  Figure 4 |