Reviewer’s report

Title: Longitudinal Study of Electrical, Functional and Structural Remodelling in an Equine Model of Atrial Fibrillation

Version: 0 Date: 01 May 2019

Reviewer: Annelies Decloedt

Reviewer's report:

This manuscript describes the use of horses as a large animal model for chronic atrial fibrillation. The horse has been previously described as a potential large animal model for atrial fibrillation, however, most studies only described short term AFib and did not include ion channel expression and myocardial tissue fibrosis analysis. Therefore, this study could be a useful addition to the existing literature.

My main remark is the power/statistical analysis of this study. Was a power calculation performed a priori or how was the sample size selected? The control group only consists of 3 horses, and the AF group of 6 horses. For example, the heart rate increases clearly during the study period but this is not significant, could this be due to lack of power? For the echocardiographic results, several measurements are missing which further decreases the sample size. This should be clarified by the authors.

The statistical analysis should also be described in more detail. As measurements over different timepoints are being assessed, longitudinal analysis seems like the most logic choice of statistical method, however, the authors also mention t-tests. It is not clear which test was used for which comparison.

Second, the AF burden seems to differ largely between the horses, making this animal perhaps less interesting as a large animal model in an experimental study? The discussion should go more into detail about this, and about possible solutions for this problem.

The AFR was measured from the surface ECG. As the horses were instrumented with leads in the right atrium anyway, wasn't it possible to measure the atrial fibrillation rate invasively from the intracardiac electrogram? Similarly, no electrophysiological measurements were included in the study, this is a major limitation.

In addition, I have some other questions and suggestions:

Line 100: How long was manual burst pacing performed? As this was done manually, I presume that it was not during hours?
Line 117: Was flecainide also administered to horses that were not in AFib? Why? Why was it also administered to the control group? Was this done to assess the effect of flecainide on the echocardiographic measurements? Were measurements in sinus rhythm in the control group also performed after flecainide administration as in the AFib group? This is not entirely clear from the manuscript. Why was the pacemaker turned off 30 minutes before flecainide treatment?

Line 236: did some horses develop mild valvular insufficiency during the study period?

Line 238-239: is it correct that the difference between SR/baseline in SR and AF was not assessed? It is not entirely clear from these sentences that there was no evolution of LV function or size throughout the AF period, however, there may have been a difference compared to the baseline in SR.

The ion channel expression methodology is included in the supplementary information but this is not clear from the manuscript. Furthermore, information is lacking about the reason why these ion channels specifically were chosen and whether changes have been demonstrated in horses in previous studies.

Fig. 3 is interesting but shows individual data of each horse and might therefore be replaced by a figure combining data of all horses.

Fig. 6: from the figure legend, the difference between A/B and D/E is not clear.

Supplementary Info:

- How do you explain the different results for LAAmax in the AF group when measured in SR vs AF? In SR, the area does not change at all, while during AF the area increases significantly.

- How do you explain the decrease of LAAa during the AF period?

Finally, the text contains several typos or grammatical errors (mainly verbs that are plural when they should be singular or vice versa). Extra proofreading is recommended.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

No

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.
Yes

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I recommend additional statistical review

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