Author’s response to reviews

Title: Investigation of changes in bone density and chemical composition associated with bone marrow oedema-type appearances in magnetic resonance images of the equine forelimb.

Authors:

Christine Heales (c.j.heales@exeter.ac.uk)
Ian Summers (i.r.summers@exeter.ac.uk)
Jonathan Fulford (j.fulford@exeter.ac.uk)
Karen Knapp (k.m.knapp@exeter.ac.uk)
C Peter Winlove (c.p.winlove@exeter.ac.uk)

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Author’s response to reviews:

Response letter has been uploaded with the revised manuscript and is also included here:

RESPONSE LETTER

Reviewer 1 Comments & Responses.

Thank you for your valuable comments which we have aimed to respond to fully, and feel has led to a significant improvement in the paper.

Study Design, Methodological Detail, Study Samples

Comment:

Details about how or why subsets of samples were collected are missing from the manuscript. For example, why was MRI only used to assess 65 forelimbs instead of 86 forelimbs from 43 horses?

Response:
It was not possible to obtain a pair of forelimbs from all animals as multiple researchers used the same source. Clarification of this point has been added to the text.

Methods, Study Samples: Line 122-123 – added text: ‘(some from a single forelimb, some from both forelimbs, dependent on availability)’

Comment:
Of 21 forelimbs had evidence of BMOA, why was radiography and Raman spectroscopy only performed on 8? Were these 8 forelimbs selected randomly out of the 21?

Response:
We recognise that the selection process was unclear within the text so this has been modified extensively.

Methods, ‘MRI Imaging’ Lines 159-166 – added / amended text: ‘The 65 limbs were then divided (chronologically) into subsets for pilot and other studies. Thirteen limbs were selected for subsequent analysis within the present study. Each limb provided two sample slices (medial and lateral – see below), 8 with BMOA present (mean age ± sd 16.0 ± 4.1 years ) and 17 controls (mean age ± sd 17.2 ± 4.1 years). One sample slice was excluded due to the presence of a cyst (see above). Of the 8 slices with BMOA present, six were obtained from limbs with both medial and lateral BMOA, and two from limbs with medial BMOA only.’

Abstract, Lines 36-37 ‘… 65 limbs from 43 horses. A subset of 13 limbs provided 25 samples, 8 with BMOA present and 17 as controls.’

Comment:
How did the demographics of the BMOA vs. non-BMOA groups differ in terms of age/breed category for the full 65 forelimb sample and for the 8 and 17 sample subsets? This information is critical for interpretation of the data and should be included as a table or tables in the manuscript. A table of how demographic/signalment variables (i.e., age, sex, breed, forelimb—left or right) varied between the BMOA group and the control group is necessary.

Response:
Given the limited element of control over the way the samples were obtained it was recognised that the study population would be heterogeneous with highly limited demographic information. As a result, the technique of ‘within slice ratio’ was intended to normalise the data between individual samples. An explanation of this has been extended within the text. Mean ages and standard deviations for each group have also been added.

Methods, ‘Raman Microspectroscopy’ Lines 237-240 amended text: ‘This was in order to reduce the influence of the heterogeneity of the sample group: variations of bone composition between animals due to factors such as age, working history and breed, effectively normalising the PLQ region to the non-lesion area of bone for each animal.’

Methods ‘MRI Imaging’ Lines 161-163: Each limb provided two sample slices (medial and lateral – see below), 8 with BMOA present (mean age ± sd 16.0 ± 4.1 years ) and 17 controls (mean age ± sd 17.2 ± 4.1 years).

Comment:

The "distal metacarpal" should be referred to as "distal metacarpal 3", at least the first time that it is referenced, and the sesamoid bones should be referred to as proximal sesamoid bones to differentiate them from the distal sesamoidean bone, or the navicular bone.

Response:

‘Third’ has been added to the text.

Methods, ‘Study Samples’ Line 129 – distal metacarpal (third metacarpal)
Methods, ‘MRI Imaging’ Line 144 – distal metacarpal (third metacarpal)
Discussion, Line 341 – dorsal third metacarpal
Figures, Line 613 – Key: a) third metacarpal bone

Sesamoid bones has been updated to read proximal sesamoid bone
Methods, Study Samples, Line 132 – proximal sesamoid bones

Figures, Line 613-614 – proximal sesamoid bone

Comment:

MRI Imaging

Methodological details are missing. What was the time frame between collecting limbs and performing the MRI? How were limbs stored prior to MRI (fresh, refrigerated, frozen)?

Response:

Details have been added to the text.

Methods, MRI Imaging. Lines 135-136 - MRI imaging was undertaken on fresh, refrigerated, limbs between 4 – 6 hours after the samples were obtained.

Comment:

How many people scored the MRI for the presence of BMOA, and was this performed by a board-certified radiologist?

Response:

Details have been added to the text.

Methods, MRI Imaging. Lines 147-148 - Image evaluation was undertaken by a single, experienced MRI radiographer (author CJH) who also performed the image acquisition.

Comment:

It is stated that T1 and T2 sequences were used to evaluate the presence of advanced osteoarthritis (OA) that would be used to exclude samples from the study. However, only one sample was excluded on the basis of this OA exclusion criteria due to the presence of a cyst (plus one sample excluded due to BMOA "felt to be due to acute trauma" and one sample excluded
due to a blood vessel). In addition, since bone cysts are typically associated with either cartilage injury and/or the development of osteoarthritis, it is not clear exactly why the sample with the cyst was excluded. More descriptive detail about the sample that was excluded because it was "felt to be due to acute trauma” would be useful.

Response:

We recognise that this section was unclear so additional details have been added to the text.

Methods, MRI Imaging Lines 154-159 - As a result, three samples were excluded as the BMOA detected was felt to be due to acute trauma (the location of the altered signal intensity being suggestive of extreme extension of the joint), a blood vessel and a cyst, respectively. The sample with the cyst was excluded as it may have been representative of advanced osteoarthritis or been a unicameral or aneurysmal cyst and may have confounded the analysis.

Comment:

Since the authors state in the abstract and discussion that the value of this study would be to investigate the relevance of BMOA relative to osteoarthritis, it seems as though it would be useful to assign an osteoarthritis score (none, mild, moderate, severe) on the basis of the MRI images in order to be able to make a statement about the relationship between BMOA and osteoarthritis. Given the frequency of osteoarthritis in the equine metacarpophalangeal joint and mean estimated age of 13.4 ± 5.4 years, it would be surprising if there weren't several samples that demonstrated some evidence of osteoarthritis.

Response:

The intention of the study was to investigate whether there are any underlying bone composition changes associated with BMOA as seen on MRI, rather than to investigate any association between BMOA, changes in bone composition (as seen with Raman microspectroscopy) and OA. The hypothesis was that BMOA might precede OA rather than be present concurrently. The text has been modified to accentuate this, and we have revised the abstract.

Abstract, Lines 55-56 - The study provides a proof of principle for the use of Raman microspectroscopy and projection radiography in in-vitro studies of BMOA.
Background, Lines 104-106 - Similarly, the Multicentre Osteoarthritis Study (MOST) study demonstrated that subchondral BMOA lesions are highly associated with, and predictive of, bone attrition in individuals who subsequently develop osteoarthritis (14).

Comment:

Raman Microspectroscopy

The methods state that a 1mm slice was cut along the sagittal plane (should clarify that this is the bone that was cut and not the fibrous joint capsule)…..

Response:

Text changed to clarify that the bone was cut. Also (in relation to the previous comments about number of samples) the text now explains that two slices were cut from each joint.

Methods, Raman Microspectroscopy, Lines 173-174 - …. two 1 mm slices through the bone were cut along the sagittal plane ……

Comment:

…..through the mid-region of the BMOA or a corresponding lesion in samples with no evidence of BMOA. However, the authors do not clarify whether BMOA were present in the medial or lateral condyle or both in each horse,

Response

Text added.

Method, MRI Imaging, Lines 164-166 Of the 8 slices with BMOA present, six were obtained from limbs with both medial and lateral BMOA, and two from limbs with medial BMOA only.

Comment:

or how close to the sagittal plane these were found. A table describing the medial/lateral distribution of BMOA lesions would be useful.
Response:

Text added.

Method, MRI Imaging, Lines 164-166 Of the 8 slices with BMOA present, six were obtained from limbs with both medial and lateral BMOA, and two from limbs with medial BMOA only.

Methods, Raman Microspectroscopy, Line 173-176- and two 1 mm slices through the bone were cut along the sagittal plane, on either side of the midline, passing through the mid-region of the BMOA lesion (when present) or in a corresponding location (typically 10 mm from the midline) when BMOA was not present.

Comment:

If the BMOA was present in only one condyle, how was the laterality of the control samples paired/matched with the BMOA samples?

Response:

Both medial and lateral samples were prepared from each limb, but the within slice ratio was used to provide the control to account for any imbalance in distribution of BMOA and any inherent variation in bone composition / density between the medial and lateral sides within an animal, as well as to control for any variations between animals.

Text has been added to clarify this:

Method, MRI Imaging, Lines 161-163 Each limb provided two sample slices (medial and lateral – see below), 8 with BMOA present (mean age ± sd 16.0 ± 4.1 years) and 17 controls (mean age ± sd 17.2 ± 4.1 years).

Method, MRI Imaging, Lines 164-166 Of the 8 slices with BMOA present, six were obtained from limbs with both medial and lateral BMOA, and two from limbs with medial BMOA only.
Comment:
How many cm of the distal portion of the 3rd metacarpal bone was dissected?

Response:
The reference to Figure 3 has been moved earlier so the reader can see how much bone was dissected.

Method, Raman Microspectroscopy, Line 172-173 - Soft tissues and ligaments were removed (Figure 3)

Comment:
How long were samples stored in 0.9% w/v saline prior to Raman microspectroscopy?

Response:
Text added to provide this information:

Method, Raman Microspectroscopy, Lines 182-184 - Raman microspectroscopy was undertaken within a week of sample preparation, typically within 24 hours.

Comment:
It would be helpful to use both the appropriate veterinary anatomical terminology, in conjunction with the human anatomical terminology, at least once to refer to the locations of the distal equine metacarpal 3 and in Figure 3.

Anterior = dorsal for this location in the horse Posterior = palmar for this location in the horse
Upper = proximal for this location in the horse Lower = distal for this location in the horse

That is, palmar osteochondral disease (POD) is known to affect the distal, palmar condyle of the equine metacarpal 3 in the veterinary literature.
Response:

Text added to address this comment:

Methods, Raman Microspectroscopy, Lines 229-231 Location names are given such that anterior would correlate to the dorsal location in the horse, posterior to palmar, upper to proximal and lower to distal anatomical locations.

Comment:

Visual Inspection

The authors state that visual inspection of the cartilage revealed minor cartilage defects in equal measure in horses with and without BMOA. What was the prevalence of minor cartilage defects in both groups? How was a minor cartilage defect defined? Were cartilage defects identified via staining with India ink? By one or several observers? Blinded or unblinded?

Response:

The discussion of cartilage lesions has been removed as it does not add value to the paper.

Results, Anatomical Location of BMOA, Lines 302-303 have been deleted. Following dissection, visual inspection of the cartilage revealed minor cartilage defects in equal measure in horses with and without BMOA.

Comment:

Reporting of Statistical Models

Details about the binary logistic regression model, including the equation and parameter estimates, should be included in the manuscript, at least as a supplement. Were other variables besides age (i.e., sex, breed) first screened for significance?

Response:

No other variables were screened for significance within the binary logistic regression model as a result of the limited demographic information available-text to this effect has been added.
Methods – Data Analysis Lines 272-274 To assess the relationship between BMOA presence and age, binary logistic regression was run. Given the lack of demographic information, no other variables were included within the analysis.

Further details of the output of the analysis have been included within the Results section.

Results, Prevalence of BMOA Lines 287-289 From binary logistic regression no significant relationship between BMOA presence and age was found (B = -0.033, ExpB = 0.967, Wald = 0.228, p = 0.630).

Comment:

Presence of OA, as identified in the MRI even if clinical lameness data is unavailable, would be an interesting and important variable to investigate with respect to the stated motivation of this study.

Response:

Please see previous comment about OA (page 3 of this response letter).

Comment:

Figures

Figure 1. Dorso-palmar (anterior-posterior) radiograph of the metacarpophalangeal joint. Should be "proximal" sesamoid bones in c.

Response:

Text amended – added ‘proximal’

Figures, Titles and Legends, Line 613-614 proximal sesamoid bones,
Medio-lateral labeling would be beneficial. Including a sagittal plane or latero-medial radiograph would be helpful to orient viewers in the same plane used to display the MRI images and the Raman microspectroscopy layout.

Response:
Figures amended – figure 2 now consists of a midline sagittal MRI image, together with a coronal to better orientate the reader.

Figure 2. A STIR and T1-weighted image of a metacarpophalangeal joint without a BMOA would be useful for comparison.

Find control image

Figure 3. The photographs describe a sagittal section. Is this truly mid-sagittal or para-sagittal? If para-sagittal, is it medial or lateral? As described in the text, the region of the distal metacarpal 3 where BMOA were present is commonly referred to as the palmar condyle or palmar osteochondral disease (POD). Both veterinary/human anatomical terminology would be useful, using the dorsal, palmar proximal and palmar distal regions for labeling.

Response:
Figure titles have been updated to state whether para- or mid-sagittal.

Figures, Line 617– Parasagittal (medial) magnetic resonance images of the metacarpophalangeal joint.

Figures, Line 624 – …mid-sagittal…..

Figures, Line 629 - …parasagittal (medial)…..

Comment:
The image in Part (a) is out of focus and a different magnification from part (b). It would be helpful to scale the images similarly and include an image in focus for part (a).
Response:

The image has been adjusted.

Comment:

Discussion

The authors state in the abstract and discussion that the horse model may be useful for future studies examining the relevance of BMOA in conditions such as osteoarthritis in humans; however, the authors provide very little evidence or motivation for why the horse model is a good model for humans or osteoarthritis in this paper. While this reviewer thinks that there is value in using the horse as a model for human disease, this manuscript is more of a proof-of-principle and does little to demonstrate how the horse is a good model for human osteoarthritis—especially when the cadaveric samples imaged are not given an osteoarthritis score on the basis of MRI, histology or any other means. This manuscript also doesn't address the very significant differences in bone area/density/volume fraction between equine and human bone. In addition, this manuscript is missing references regarding the well-established phenomenon in horses referred to as traumatic osteochondrosis or palmar osteochondral disease (POD). The authors include two references to textbooks on equine joint or bone disease in references 19 and 28 (page numbers are missing), but don't include any references to the primary literature describing traumatic osteochondrosis/POD in horses.

Response:

It is acknowledged that the paper represents a proof-of-principle approach, and agree that future studies require further detailed information about the equine history, and detailed evaluation e.g. of cartilage lesions, histology etc. It is also recognised that there will be differences between equine and human pathophysiology. The text has been amended to reflect this.

Title page – Line 2-4: Investigation of changes in bone density and chemical composition associated with bone marrow oedema-type appearances in magnetic resonance images of the equine forelimb.
Abstract, ‘Background’ – Line 31-33 - …. aimed to investigate the potential of projection radiography and Raman microspectroscopy to provide information ……

Abstract, Line 55-56 – reference to use of horse model in humans deleted and amended to …. The study provides a proof of principle for the use of Raman microspectroscopy and projection radiography in in-vitro studies of BMOA.

Discussion, Lines 423-426 Whilst there are differences between equine and human bone it is also felt that there are similarities, for example in the pathogenesis of osteochondrosis (40), that may also render these findings applicable to the human population.

Conclusion Lines 438-442 The study provides a proof of principle for the use of Raman microspectroscopy and projection radiography in in-vitro studies of BMOA. These techniques may be a useful adjunct for further investigations into the pathophysiology of equine joint disease, which may have some relevance to similar conditions in the human population.

Comment:

In addition, reference 28 refers to microfractures within the dorsal metacarpal bone, which is less pertinent to the topic of interest than the extensive literature reporting microdamage accumulation in the distal metacarpal 3 adjacent to the BMOA reported in this manuscript in horses with and without condylar fractures. Primary literature relevant to traumatic osteochondrosis/POD phenomenon in horses can be found by Christopher Whitton, Christopher Riggs, and John Peloso's groups, including literature describing the appearance of BMOA in the distal metacarpal 3 of horses that sustained condylar fractures as compared to controls (Peloso et al. 2019). The authors also state that the BMOA present in this study were unlikely to be a form of traumatic osteochondrosis/POD as BMOA were not shown to be associated with damage to the articular cartilage; however, some studies suggest that bone damage may precede the development of articular cartilage damage, and this should be addressed.

Response:

The discussion has been updated to reflect this.

Discussion, Location of BMOA, Lines 337-339

Comment:
One of the major limitations with this manuscript is that few relevant clinical details are available for the post-mortem samples imaged in this study, including details about (accurate) age, breed, lameness, clinical history and exercise history. This significant limitation should be adequately addressed in the discussion. The authors state that experienced abattoir staff aged horses (34 out of 43 horses), but the aging of horses via dental examination is not very accurate in older horses. In addition, because lameness data is not available for these horses, the authors cannot correlate the appearance of BMOA in this cohort with evidence of lameness, which would allow the authors to make a stronger statement about the usefulness of these modalities for studying osteoarthritis in humans. Osteoarthritis is quite common in the equine population, and it is surprising that, in a population with a mean estimated age of 13.4 ± 5.4 years, that there was no evidence of osteoarthritis detected on MRI—or perhaps this was not assessed, with the exception of "severe" osteoarthritis. Finally, the signalments of the three groups, including yearlings, riding school horses and ponies, and wild Dartmoor ponies are very diverse populations and would be expected to have very different prevalences for osteoarthritis or BMOA. Thus, a table reporting the presence of BMOA relative to each of these breed/age groups and relative to the subsets of samples selected for projection radiography and Raman spectroscopy is critical for the interpretation of this manuscript.

Response:

Clarification added that dental ageing of horses is not precise which has been included within a new Limitation section, which covers a range of points raised by the reviewer.

Methods, Study Samples Line 127-128 - this provides an estimation of age, although it is not an entirely precise method

Limitations Lines 397-406 - The study represents only a small-scale assessment of the use of horse-bone sources to examine BMOA. In addition, the sample population used for these studies was heterogeneous, with very limited demographic information. Hence it was not possible to consider the effect on the measured data of horse breed, sex or working history. It was possible to consider the effect of horse age (see above), but these data require cautious interpretation as ageing a horse using dental examination is not precise (18). Furthermore, the study did not attempt to evaluate or grade the tissue samples for osteoarthritis. However, despite these limitations, the study provides evidence that differences in bone density are associated with BMOA, suggesting that the techniques of the present study may provide useful avenues for further exploration.
Comment:

Future work

The authors suggest that additional Raman spectroscopy could be used to examine whether BMOA lesions were associated with microdamage; however, histology or SEM would provide significant complementary value to the Raman data in terms of demonstrating cracks, microfracture, and histological evidence of cell and extracellular matrix/damage remodeling.

Response:

This has been added as suggestions for future work.

Discussion, Future work Lines 420-423 Combining Raman microspectroscopy in a controlled equine population, alongside techniques such as histology or Scanning Electron Microscopy would enable information about bone remodelling to be correlated with any demonstration of micro-fracture, cracks and histological evidence of bone remodelling.

Reviewer 2 (Reviewer 2): PEER REVIEWER ASSESSMENTS:

Thank you for your helpful comments which we have addressed below.

REQUESTED REVISIONS:

Comment:

Please specify how you localized the specimens to analyze for Raman spectroscopy from the MRI.

Response:

Clarification added to the text:

Methods, Raman Microspectroscopy Lines 171-179
It would have been helpful to exam the histology of the specimens to characterize the presence of osteoid and osteoblasts in areas of edema.

Response:

It was not possible within the scope of this project to undertake this type of histology.

Comment:

The MR images are rather grainy and the arrows on figure 2 seem to be a bit far from the area of edema.

Response:

The region of bone marrow oedema is not uniform but becomes less prominent with distance from the bone surface. The arrows are felt to depict the full limit of the bone marrow oedema (particularly when viewed on a dedicated medical imaging display screen). The STIR images typically appear grainy due to low signal, as this is a ‘low fluid’ region of anatomy. An inversion time of 150ms was used to ensure maximum suppression of signal from fat, which also contributes to the images appearing noisy.

Comment:

There are a few typos, like "This suggest" instead of "This suggests" on line 424.

Response:

This has been corrected.

Comment:

You may want to add that a p-value of 0.05 was used to define statistical significance and add the p-values of the pertinent results in the abstract.
Response:

Thank you for your suggestion; we have amended the abstract as suggested, and stated the critical p value in line 311-312 and in the abstract at line 42-43