Osteoarthritis is a major clinical problem in many species, including horses. Once it is initiated, it is irreversible and progressive.\textsuperscript{1,2} Osteoarthritis can develop acutely after a single traumatic incident or chronically through a series of smaller concussive forces.\textsuperscript{3} The inability of hyaline articular cartilage to completely heal leads to accumulated damage, which increases the prevalence of clinically apparent disease in aging subjects.\textsuperscript{4–6} The incidence of clinically apparent osteoarthritis is not equal among all joints. Although certain changes accumulate with age, changes can also occur in the absence of clinically apparent osteoarthritis.\textsuperscript{6} The incidence of osteoarthritis of the TMJs in humans increases from approximately 25% in those 20 to 49 years old\textsuperscript{7} to 70% in those 73 to 75 years old.\textsuperscript{8} Interestingly, only mild pain and clinical signs of osteoarthritis were found in the latter group, which supports suggestions that osteoarthritis of a TMJ causes pain in the early stages but causes less pain as it progresses. Multiple histologic grading systems have been used to evaluate osteoarthritis and age-related changes in hyaline cartilage joints as well as the TMJs.\textsuperscript{9–20}

The arrangement of the collagen fiber apparatus on the articular surfaces of equine TMJs has been described.\textsuperscript{21} The collagen fibers are differentially oriented to correspond with the complicated movement of the joints. The same research group subsequently published a comprehensive histologic description of the TMJs of horses;\textsuperscript{22} however, to our knowledge, there has been no description of the accumulation of histologic changes within these joints as horses age. Therefore, the objective of the study reported here was to describe histologic changes in the TMJs of a sample of clinically normal horses of various ages. The hypothesis was that as horses age, the TMJs would develop signs of age-related change. Results of a histologic assessment of age-related changes in the TMJs of horses will help determine whether there is age-related degeneration of these joints that potentially could lead to osteoarthritis.

Materials and Methods

Sample
The heads of 11 equine cadavers were used in the descriptive study reported here. The cadavers represented a convenience sample of client-owned

Abbreviations
TMJ Temporomandibular joint

OBJECTIVE
To describe histologic changes in the temporomandibular joints (TMJs) of horses of various ages.

SAMPLE
22 TMJs from cadavers of 11 horses.

PROCEDURES
Horses were categorized into 3 age groups (group 1, 2 to 10 years old [n = 3]; group 2, 11 to 20 years old [3]; and group 3, > 20 years old [5]). Each TMJ was sectioned into 5-mm slices, preserved in formalin, decalcified in formic acid, and routinely processed for histologic analysis. Joints were systematically assessed by use of previously described methods. Multilevel mixed-effects models were used to examine the data.

RESULTS
The number of changes was significantly fewer and degree of changes was significantly less within the TMJs of group 1 horses, compared with those of group 3 horses. Comparison among groups revealed that the combination of temporal and mandibular scores for group 1 was significantly lower than for groups 2 or 3. Disk score did not differ significantly between groups 1 and 2, but disk scores of groups 1 and 2 were significantly lower than the disk score of group 3.

CONCLUSIONS AND CLINICAL RELEVANCE
The assessed lesions were associated with osteoarthritis, and they accumulated in the TMJs as horses aged. In the absence of signs of pain manifested as changes in mastication, behavior, or performance, it would be difficult to determine the point at which accrued pathological changes represented the onset of clinically important osteoarthritis of the TMJs. (Am J Vet Res 2019;80:1107–1113)
horses euthanized for reasons unrelated to dental, TMJ, or head disease. Horses comprised 5 geldings and 5 mares (sex of 1 horse was not recorded) and were 2 to 32 years old as determined from the medical records. Reasons for euthanasia were request of the owners (n = 5), severe wounds to the distal aspects of the limbs resulting in the compromise of synovial structures (2), severe weight loss (not related to dentition; 1), recumbency with inability to stand (1), and advanced arthritis of the distal interphalangeal joint (1); the reason for euthanasia was not recorded for 1 horse. Medical records were further examined to ensure the horses did not have difficulties in prehension or mastication of food. All owners provided consent for collection of specimens for use in the study.

Experimental procedures

Horses were categorized into 3 groups on the basis of age. Group 1 consisted of horses 2 to 10 years old (n = 3), group 2 consisted of horses 11 to 20 years old (3), and group 3 consisted of horses > 20 years old (5). Heads were examined, including palpation of the masseter and temporalis musculature as well as the skeletal margins of the TMJs, immediately after horses were euthanized. After the postmortem examination was completed, the heads were frozen at −20°C for 5 days. Both TMJs of each frozen head were sectioned in 5-mm slices by use of a band saw. The TMJs of each head were arbitrarily selected to be sliced in a sagittal or transverse orientation. Each sectioned TMJ was stored separately in neutral-buffered 10% formalin until fixation was completed. Soft tissues were removed, and each TMJ was disarticulated. Articular disks were sectioned and processed (ie, embedded in paraffin, cut at a thickness of 4 µm, mounted on glass slides, stained with H&E stain, and covered with a cover slip) for routine microscopic examination. Bones of each TMJ were placed in 20% formic acid until they were decalcified; they then were processed in a similar manner for routine microscopic examination.

Slices of articular disks and bones were assigned a region number (from 1 to 6 [lateral to medial] in sagittal sections and from 1 to 5 [rostral to caudal] in transverse sections) to enable systematic identification of all samples. All components of the TMJs were assessed histologically by a board-certified veterinary pathologist (ALA) by use of methods previously applied to the TMJs of mice and rabbits. The pathologist was not aware of the source of each sample.

Each region consisted of 3 slides. The temporal components of a TMJ (retroarticular process, mandibular fossa, and articular tubercle), the mandibular condyle, and the articular disk were examined (between 4X and 60X magnification) to assess vertical clefts, regions of acellularity, chondrocyte clusters, and large chondrones (Figure 1). A large chondrone was defined as ≥ 6 chondrocytes in a cluster. Each characteristic in each slide was graded (score of 0 to 2), which yielded a total condylar score of each region that ranged from 0 to 6 (Appendix). The articular disks were examined to detect a decrease in cellularity, scattered individual metaplastic chondroid cells, ≥ 1 focus of chondroid metaplasia, and ≥ 1 focus of osseous metaplasia (Figure 2). Each characteristic was scored as present (1) or absent (0), which

![Figure 1](https://via.placeholder.com/150)

Figure 1—Photomicrographs of tissue sections of articular cartilage from the temporal articular tubercle and the mandibular condyle of horses of various ages. A—Section from a 2-year-old horse with minimal changes. Notice the large number of evenly distributed chondrocytes. B—Section from a 2-year-old horse with a few areas of acellularity (ovals) surrounded by large numbers of evenly distributed chondrocytes. C—Section from a 14-year-old horse with moderate numbers of evenly distributed chondrocytes (rectangles) surrounded by moderate numbers of evenly distributed chondrocytes. D—Section from a 14-year-old horse with multiple and coalescing areas of acellularity (ovals) and 1 area of clustered chondrocytes (rectangle). Notice the general reduction in the number of chondrocytes. E—Section from a 32-year-old horse with extensive areas of acellularity (ovals) and a few clustered chondrocytes (rectangles). F—Section from a 32-year-old horse with several examples of large chondrones (rectangles); a large chondrone was defined as ≥ 6 chondrocytes in a cluster. H&E stain; bar = 100 µm.
yielded a total disk score for each region that ranged from 0 to 4. Total score for each TMJ region was the sum of the scores for each of the 3 factors (ie, temporal components of a TMJ [range, 0 to 6], mandibular condyle [range, 0 to 6], and articular disk [range, 0 to 4]); therefore, total score for each TMJ region ranged from 0 to 16. Because 1 TMJ for each horse was sectioned in the sagittal plane (n = 6 regions) and 1 TMJ was sectioned transversely (5 regions), the TMJ sectioned in the sagittal plane had a score ranging from 0 to 96 (6 sections with a maximum score of 16/section), and the TMJ sectioned in the transverse plane had a score ranging from 0 to 80 (5 sections with a maximum score of 16/section). The total score for each horse was the sum of the region scores for both TMJs (11 sections with a score ranging from 0 to 16/section = maximum total possible score of 176).

### Statistical analysis

Linear regression analysis within a multilevel mixed-effects model was used to examine the effects of age group on total horse, total TMJ, total condyle, and total disk scores. Normality of residuals was assessed with the Shapiro-Wilk test. Ordinal logistic regression within a multilevel mixed-effects model was used to examine the effect of age group on scores of the individual variables of the temporal components of a TMJ, mandibular condyle, and articular disk. Within this model, horse, side of head, anatomic location (temporal components, mandibular condyle, and articular disk), and region were fixed effects. Because there was a higher number of regions in the sagitally versus transversely sectioned planes, plane of section (sagittal or transverse) was included as a random effect. Values were considered to differ significantly at \( P < 0.05 \).

### Results

None of the 6 horses admitted to the clinic for examination and treatment had evidence in the medical record of problems with prehension or mastication of feed. Postmortem examinations revealed that none of the 11 horses had obvious abnormalities or asymmetries of the masticatory musculature or TMJs. Complete oral examinations were not performed. Gross examination of the TMJs was not performed because it would have necessitated opening of the joints, which would have disrupted the relationships among the joint components. Instead, the TMJs were frozen to ensure that the articular surfaces and disk remained opposed and immovable during sectioning with the band saw.

Total horse score and total TMJ score did not differ significantly (\( P = 0.7 \)) between male and female horses. Overall, there was no significant difference in total joint score (\( P = 0.4 \)), total condylar score (\( P = 0.1 \)), or total disk score (\( P = 0.8 \)) between the left and right joints in each horse. There was a significant (\( P = 0.003 \)) difference in total TMJ score among age groups. Horses in group 1 had a significantly (\( P = 0.002 \)) lower total TMJ score than did horses in group 3; however, there was no significant difference in total TMJ score between horses of groups 1 and 2 (\( P = 0.2 \)) or groups 2 and 3 (\( P = 0.7 \)). Total scores for the combination of the temporal components and mandibular condyle differed significantly (\( P < 0.001 \)) among groups; total scores differed significantly between groups 1 and 2.

![Figure 2](image-url)
with age, which was not surprising. However, there is sparse information regarding the role of age-related changes in the joints of horses, and the authors are not aware of any information specifically describing age-related changes of the TMJs. The findings of the present study will add to the growing body of evidence that the TMJs of horses are dynamic joints.

The modified Mankin scoring system has been widely used in the histologic assessment of osteoarthritis in humans and other animals. The Osteoarthritis Research Society International histopathology initiative has developed individual scoring systems for animals, including rats and horses among others, used in the study of osteoarthritis. Although these systems were developed for use in hyaline cartilage joints, they have been applied to the fibrocartilaginous TMJs of rabbits; initial examination of the histologic specimens of rabbits revealed pathological features of osteoarthritis. We elected to apply a scoring system to the equine TMJs that was based on the same wide-ranging histopathologic features of osteoarthritis reported in another study. Initial formation of vertical clefts was chosen as a possible scoring characteristic because of its presence in TMJs of other species. However, vertical clefts were not observed in the equine TMJs of the present study. It is possible that, in contrast to the situation in other species, the presence of vertical clefts is an insensitive measure of age-related change in the TMJs of horses. This theory is supported by results of another study that involved examination of CT images of the TMJs of > 1,000 horses, only 0.7% of which had grossly evident vertical clefts. The low prevalence of this characteristic may suggest that cleft formation within the condyles is restricted to the process of osteoarthritis and is not seen in clinically normal horses.

The TMJs undergo adaptive remodeling, and the intra-articular environment changes as horses mature. The role of TMJ disorders, specifically osteoarthrosis, have yet to be fully elucidated in horses. Clinical osteoarthrosis of the TMJs reportedly has an effect on performance of sport horses; thus, it is possible that subclinical osteoarthrosis of the TMJs also has a similar effect. However, the lack of clarity between the characteristics of age-related change and characteristics of osteoarthritis makes it difficult to separate the 2 processes and determine the clinical effects of each. Investigators of experimentally induced osteoarthritis use many of the same grading systems for animals, including rats and other animals.

Scores for variables differed significantly among age groups when data were categorized on the basis of the anatomic location of the histologic section (Supplementary Tables S1 and S2, available at avmajournals.avma.org/doi/suppl/10.2460/ajvr.80.12.1107). There were no vertical clefts seen in any histologic preparations.

**Discussion**

Findings for the study reported here indicated that the TMJs of horses accrued histologic changes

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**Figure 3**—Box-and-whisker plots of the total horse score for the TMJs of horses of various ages. Group 1 consisted of horses 2 to 10 years old (n = 3), group 2 consisted of horses 11 to 20 years old (3), and group 3 consisted of horses > 20 years old (5). Total score was calculated for each horse (total score ranged from 0 to 176) as the sum of the scores for both TMJs in a horse; total score for each TMJ was calculated as the sum of the scores for each of the 3 anatomic portions of the joint (ie, temporal components of a TMJ, mandibular condyle, and articular disk) in each of 6 (sagittally sectioned), or 5 (transversely sectioned) regions. Each box represents the 25th to 75th percentiles, the horizontal line in each box represents the median, the whiskers represent the minimum and maximum values, and the circle represents an outlier that is the result for a 32-year-old horse that was substantially older than the other horses in the study.

(P = 0.03) and groups 1 and 3 (P < 0.001) but not between groups 2 and 3 (P = 0.4). Total disk score differed significantly (P < 0.001) among age groups; total disk score differed significantly between groups 1 and 3 (P < 0.001) and groups 2 and 3 (P = 0.02) but not between groups 1 and 2 (P = 0.05).

Similarly, total horse score (sum of the scores for the left and right TMJ) differed significantly (P = 0.01) among age groups (Figure 3). Total horse score differed significantly between groups 1 and 3 (P = 0.01) but not between groups 1 and 2 (P = 0.8) or groups 2 and 3 (P = 0.2). Total horse score did not differ significantly (P = 0.4) on the basis of section orientation (sagital vs transverse). However, joint score differed significantly (P = 0.005) between section orientation, with TMJs sectioned sagittally having a greater mean value (14.85) than did those sectioned transversely (6.67).

Scores for variables differed significantly among age groups when data were categorized on the basis of the anatomic location of the histologic section (Supplementary Tables S1 and S2, available at avmajournals.avma.org/doi/suppl/10.2460/ajvr.80.12.1107). There were no vertical clefts seen in any histologic preparations.

**Discussion**

Findings for the study reported here indicated that the TMJs of horses accrued histologic changes...
in the present study (eg, chondrone formation and chondro-osseous metaplasia) were also observed in a horse of another report. Therefore, it is possible that the characteristics described as disease-related changes in that case report were an incidental finding and typical for a horse of that age (18 years old). The only difference between the present study and that case report was the description of osteophytes in the horse of the case report, which may suggest that the presence of osteophytes is a characteristic that separates age-related change from disease-related osteoarthritis. Contrary to this supposition is a report that indicates the presence of medial periarticular osteophytosis is a function of age and not disease. However, this statement was based on the evaluation of CT images and not on histologic assessment of the TMJs.

Total TMJ score did not differ significantly between left and right joints within an age group for the horses of the present study. This is in agreement with results of other studies and is not surprising because horses appear to have the ability to masticate interchangeably using both sides of the mandible. Compared with young horses, older horses had more frequent regions of acellularity and chondrone formation in both the temporal components (retroarticular process, mandibular fossa, and articular tubercle) and mandibular condyles. Strikingly similar histologic trends have been found in the TMJs of humans and mice.

The articular disks of older horses also had a decrease in cellularity, chondroplasia metaplasia, and chondro-osseous metaplasia, compared with results for younger horses. The horses in group 1 (the youngest horses) had significantly fewer total joint changes than did horses in group 3 (the oldest horses); however, results for these 2 groups did not differ significantly from findings for the horses of group 2. The continued accumulation of histologic changes in TMJs as horses aged suggested that the fact we did not detect significant differences among all 3 groups may likely have been attributable to the low power of the study (ie, a small number of horses in each age group). Age-related changes in the TMJs and knee joints of humans include an increase in calcification in aging patients. However, another author noted that both calcium deposition and vertical cleft formation were only seen in arthritic joints.

One possible limitation of the study reported here was that no attempt was made to assess the correlation between histologic score and inflammatory cytokine concentrations, which may have allowed us to ascertain the importance of the histologic changes. Another possible limitation was that oral examinations were not performed; however, evidence suggests that the interrelationship between dental and TMJ abnormalities is limited. Furthermore, the risk of dental pathological conditions increases with age; therefore, determining the portion of histologic changes of TMJs associated with age, versus that associated with concurrent dental disease, would have been extremely difficult.

Irrespective of these limitations, results of the study reported here suggested that clinically normal horses accumulated changes in the TMJs with age, which were similar to those seen in the TMJs of other species. Further studies are needed to validate the scoring system and describe the observed histologic changes in horses with clinically apparent TMJ disease. An assessment of the correlation between histologic and biochemical age-related changes in the TMJs is also required.

Acknowledgments

The authors declare that there were no conflicts of interest.

Footnotes

b. STATA, version 13, StataCorp LLC, College Station, Tex.

References


The appendix appears on the next page
### Appendix

Scoring criteria for histologic assessment of the TMJs of horses of various ages, by anatomic location (region).

<table>
<thead>
<tr>
<th>Anatomic location</th>
<th>Variable</th>
<th>Description</th>
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<tr>
<td>Temporal components</td>
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<td>of TMJ</td>
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<tr>
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<td></td>
<td>Coalescing</td>
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<td>Chondrocyte clusters</td>
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<tr>
<td></td>
<td></td>
<td>Rare</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Several</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Large chondrones*</td>
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<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rare</td>
<td>1</td>
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<tr>
<td></td>
<td></td>
<td>Several</td>
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<tr>
<td></td>
<td><strong>Total temporal</strong></td>
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<td><strong>Total region score</strong></td>
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Total condylar score (temporal components of TMJ or mandibular condyle) ranged from 0 to 6, and total disk score ranged from 0 to 4; thus, total score for each region of each TMJ ranged from 0 to 16 because each TMJ consists of 2 condyles (temporal and mandibular) and a disk.

*A large chondrone was defined as ≥ 6 chondrocytes in a cluster.