Received: 9 October 2021 | Revised: 4 January 2022 | Accepted: 17 January 2022

DOI: 10.1111/vco.12802

ORIGINAL ARTICLE

Veterinary and Comparative Oncology WILEY

Standardisation of canine meningioma grading: Inter-observer agreement and recommendations for reproducible histopathologic criteria

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Funding information
The authors disclosed the following financial support for the research, authorship, and/or publication of this article: Impulsion Santé Globale 2019 grant from VetAgro Sup, Lyon.

Abstract

The human grading system is currently applied to canine meningioma, although it has not been validated in dogs. The present study focused on standardising the human grading system applied to canine meningioma. Four veterinary neuropathologists graded 186 canine meningiomas as follows: Grade I tumour, with <4 mitoses/2.37 mm^2; Grade II tumour, with ≥4 mitoses/2.37 mm^2, brain invasion or at least three of the following criteria: sheeting architecture, hypercellularity, small cells, macronucleoli, necrosis; Grade III tumour, with ≥20 mitoses/2.37 mm^2 or anaplasia. Slides with grading disagreement were reviewed to define a consensus diagnosis and to assess reproducible criteria. Concordance between histologic grade and the consensus diagnosis, as well as intra- and inter-observer agreements for each criterion, were statistically analysed. Concordance between histologic grade and consensus diagnosis ranged from 59% to 100%, with lower concordance for Grade I and II tumours. The lowest inter-observer agreement was recorded for macronucleoli, small cells, hypercellularity and sheeting architecture. Tumour invasion and necrosis displayed fair agreement, while moderate agreement was reached for mitotic grade and anaplasia. The following recommendations were issued to improve the reproducibility of canine meningioma grading: (1) Assess mitotic grade in consecutive HPFs within the most mitotically active area; (2) Define invasion as neoplastic protrusions within central nervous tissue without pial lining; (3) Report spontaneous necrosis; (4) Report prominent nucleoli when visible at ×100; (5) Report pattern loss when visible at ×100 in >50% of the tumour; (6) Report necrosis, small cells, hypercellularity and macronucleoli, even when focal; (7) Report anaplasia if multifocal.

KEYWORDS
central nervous system, dog, grading, meningioma, standardisation

Sara Belluco and Giuseppe Marano contributed equally to this study.

1 | INTRODUCTION

Canine meningioma is one of the most commonly diagnosed tumours in the central nervous system, representing up to 51.5% of intracranial tumours diagnosed at necropsy.[1,2] Intracranial and spinal meningiomas produce a variety of clinical signs including seizures, altered mentation, ataxia and paresis.[1,3–5] The median survival time is more than 1 year following surgical resection and increases if adjunctive radiotherapy is applied.[4,6–8]

Due to the striking pathological, immunological and MRI similarities between human and canine meningiomas,[9] and since the veterinary WHO classification is outdated,[10] canine meningioma can be classified into three grades according to the 2016 WHO human histological grading system.[11–15] Currently, no study has addressed the translatability/transferability of the human grading system to the canine patient, in terms of accuracy and reproducibility.

In order to standardise a grading system for canine meningioma, the specific goals of this study are: (1) to evaluate veterinary neuropathologists' inter-observer agreement when applying the human grading system to canine meningioma; (2) to evaluate the concordance of each neuropathologist's grading to a consensus diagnosis; (3) to evaluate the reproducibility of each evaluated histologic criterion, in order to identify its impact in creating histologic grade disagreement; (4) to propose useful amendments to the grading applied to canine meningioma in order to increase reproducibility.

2 | MATERIAL AND METHODS

2.1 | Samples

The minimum sample size was calculated using a 95% confidence interval for the Kappa index in the presence of multiple raters and multinomial outcomes, with a precision of 10%.[16] The expected a priori agreement was considered 90%[17]; the number of veterinary neuropathologists (defined as observers) was five and the expected frequencies, based on the relative frequencies of meningioma in the laboratory archive of the participants, were 54%, 37%, 9% for Grade I, II and III, respectively.

The protocol was reviewed and approved by the ethical committee of VetAgro Sup. For each grade, a representative number of paraffin-embedded samples of canine meningioma was selected by two veterinary pathologists with over 20 years of expertise in neuropathology and retrieved from their laboratory archive. For each tumour, a 4-μm-thick section was stained with haematoxylin and eosin. The slides were digitised with an NDP scanner (NDP scan 2.5.90, Nanozoomer HT, Hamamatsu) with a magnification of ×20 (454 nm/pixel) and visualised with the free NDP.2 viewer (NDP.view2 Viewing software U12388-01|Hamamatsu Photonics).

2.2 | Histologic evaluation of the tumours

Each case was analysed twice, with an interval of at least 2 weeks between analyses, by four board-certified veterinary pathologists with a track record of training and employment in neuropathology laboratories and with extensive publication in the field (from now on defined as neuropathologists). The previous consensus on histologic criteria was not assessed. The neuropathologists defined the tumour subtype, the mitotic grade and the histologic grade for each case. The mitotic grade, purely based on the number of detected mitoses, was calculated as follows: mitotic grade 1: tumours with <4 mitoses in 2.37mm^2; mitotic grade 2: tumours with a mitotic count between 4 and 20 in 2.37mm^2; mitotic grade 3: tumours with ≥20 mitoses in 2.37mm^2. The histologic grade, based on cellular morphology, architecture and mitotic count, was evaluated as follows: Grade I: tumours lacking histologic criteria of Grade II and Grade III; Grade II: tumours with a mitotic grade of 2 or tumours with central nervous tissue invasion or tumours displaying at least three of the following criteria: sheeting architecture, small cells, hypercellularity, macronucleoli and spontaneous necrosis; Grade III: tumours with extreme anaplasia and/or tumours with a mitotic grade of 3. Since canine meningioma subtypes have not been definitely correlated to prognosis, the tumour subtype was not considered for the histologic grade.

For each slide, the tumour area of the largest sample fragment was calculated by each pathologist, using NDP view.2 free software.

The consensus diagnosis for the histologic grade and the mitotic grade was obtained by reviewing the cases with histologic disagreement by all the participants at roundtables. Consensus diagnosis was defined as the diagnosis given by at least three out of four pathologists (majority consensus diagnosis).

2.3 | Statistical analysis

According to the methods used to calculate sample size,[16] the inter-observer and the intra-observer agreements were estimated using respectively the Fleiss' Kappa index and the Cohen's Kappa coefficient. The concordance correlation coefficient (CCC_{TOT}), accounting both inter- and intra-observer agreement, the CCC_{INTER}, measuring the inter-observer agreement and CCC_{INTRA}, measuring intra-observer agreement, were calculated. For each of the three indices, a unilateral 97.5% lower confidence limit was calculated.

For histologic and mitotic grades, the concordance between each observer's second reading classification and the consensus diagnosis was calculated by evaluating the accuracy, quantifying the number and respective percentage of the observer's classifications that matched the consensus diagnosis.

In order to evaluate the possible impact of the histologically available tumour size on agreement about the histologic and mitotic grade, estimates of CCC_{TOT}, CCC_{INTER} and CCC_{INTRA} were calculated separately for samples measuring arbitrarily less than or equal to 10 mm^2 (small samples) and for samples of more than 10 mm^2 (regular samples).

Analyses were performed using R software version 4.0.4[18] with the Agreement[19] and irrCAC[20] packages added, and the Knime Analytics Platform release 4.2.3.[21,22] For κ and CCC-based agreement evaluation, the classification given by Landis and Koch[23] was used: κ

from 0.00 to 0.20 for slight agreement; from 0.21 to 0.40 for fair agreement; from 0.41 to 0.60 for moderate agreement; from 0.61 to 0.80 for substantial agreement; and over 0.80 for almost perfect agreement.

3 | RESULTS

3.1 | Samples

The calculated minimum sample size was 168, which was increased up to 186 samples (+10%) to account for potential sample losses related to technical issues. Since one of the five neuropathologists previously recruited for slide evaluation was involved in selecting the tumour samples, he was excluded from the observer group. The confidence interval precision for the Fleiss' Kappa recalculated on four observers was 10.8%, which was considered a negligible loss due to the reduction in number of observers.

3.2 | Inter-observer agreement

For histologic grade, the inter-observer agreement was moderate at the first and second reading (first reading: $\kappa = 0.52$, 95% CI: 0.42–0.61; second reading $\kappa = 0.52$, 95% CI: 0.42–0.61). At the first reading, 66/186 samples (35.9%) received the same histologic grade by the four neuropathologists: 26 of these were classified as Grade I, 33 as Grade II and 7 as Grade III. At the second reading, 73/186 samples (39.7%) received the same histologic grade by the 4 neuropathologists; 29 of these were classified as Grade I, 36 as Grade II and 8 as Grade III. More details are provided in Table S1.

For histologic grade, CCC_{TOT} accounting for intra- and inter-observer agreement was 0.52 (one-sided 97.5% CI: 0.42–1) indicating moderate agreement (Table 1). The CCC_{INTRA} was almost perfect (0.91, one-sided 97.5% CI, 0.88–1), while the CCC_{INTER} was moderate (0.54, one-sided 97.5% CI, 0.45–1).

3.3 | Concordance with consensus diagnosis

In the slide review process, majority consensus was achieved on the histologic grade in 156/186 samples (83.9%) and on mitotic grade in 135/186 samples (72.6%). For the histologic grade, 73/156 (51.7%), 72/156 (41.1%) and 11/151 (7.3%) samples were classified as Grade I, II and III, respectively. For the mitotic grade, 92/135 (68.1%), 34/135 (34.8%) and 9/135 (6.7%) were classified as Grade I, II and III, respectively. Each neuropathologist's concordances of histologic grade to the consensus diagnosis at the second reading are reported in Table 2. For histologic grade, the total accuracy ranged from 72.4% to 90.3%: the highest accuracy was found for Grade III, with agreement ranging from 9/11 (81.8%) to 11/11 (100.0%) of samples, while the percentages of concordant diagnosis ranged from 58.9% to 90.4% for Grade I and from 73.6% to 93.1% for Grade II.

Similar results were obtained for the mitotic grade (Table 3). The total accuracy between a neuropathologist's second reading and the consensus diagnosis ranged from 82.2% to 95.6%. The highest concordance was recorded for Grade III, with the percentage of concordant diagnosis close to or equal to 100.0% (except for observer C). For Grade I, the percentage of agreement ranged from 85.9% to 97.8%, while for Grade II the range was from 67.6% to 88.2%.

3.4 | Histologic criteria

Considering each histologic evaluated criterion, the agreement was moderate for mitotic grade and extreme anaplasia ($CCC_{TOT} = 0.51$ for both) and fair for tumour invasion and spontaneous necrosis ($CCC_{TOT} = 0.43$ and 0.44, respectively) (Table 1). For each of these four criteria, the intra-observer agreement was almost perfect (CCC_{INTRA} ranging from 0.86 to 0.93), while the inter-observer agreement was moderate (CCC_{INTER} ranging from 0.44 to 0.55).

The agreement was slight to fair for macronucleoli, small cells, hypercellularity and sheeting architecture (CCC_{TOT} ranging from 0.18 to 0.34) (Table 1). For these histologic criteria, intra-observer

TABLE 1 Estimates of the concordance correlation coefficients for histologic grade and histologic criteria

Index	Histologic grade	Mitotic grade	Tumour invasion	Necrosis	Macro nucleoli	Small cells	Hyper cellularity	Sheeting architecture	Extreme anaplasia
CCC_{TOT}	0.52 (0.42)	0.51 (0.40)	0.43 (0.33)	0.44 (0.36)	0.34 (0.27)	0.23 (0.15)	0.18 (0.13)	0.30 (0.23)	0.51 (0.36)
CCC_{PREC}	0.52 (0.43)	0.52 (0.41)	0.47 (0.38)	0.45 (0.37)	0.38 (0.32)	0.25 (0.17)	0.27 (0.22)	0.36 (0.29)	0.53 (0.40)
χ	0.99 (0.97)	0.98 (0.96)	0.91 (0.86)	0.98 (0.96)	0.88 (0.84)	0.91 (0.85)	0.66 (0.59)	0.83 (0.76)	0.97 (0.91)
CCC_{INTER}	0.54 (0.45)	0.55 (0.44)	0.44 (0.34)	0.47 (0.39)	0.37 (0.29)	0.26 (0.17)	0.19 (0.14)	0.33 (0.25)	0.53 (0.39)
CCC_{PREC}	0.55 (0.46)	0.56 (0.45)	0.49 (0.40)	0.48 (0.40)	0.42 (0.35)	0.29 (0.20)	0.31 (0.25)	0.40 (0.33)	0.55 (0.42)
χ	0.99 (0.96)	0.98 (0.95)	0.90 (0.86)	0.98 (0.95)	0.87 (0.83)	0.90 (0.83)	0.63 (0.56)	0.81 (0.74)	0.96 (0.90)
CCC_{INTRA}	0.91 (0.88)	0.87 (0.83)	0.93 (0.90)	0.86 (0.82)	0.83 (0.78)	0.70 (0.65)	0.77 (0.71)	0.78 (0.73)	0.90 (0.84)

Note: Estimates of the total, inter-observer and intra-observer concordance correlation coefficients and respective sub-indices (precision and accuracy) for each histologic parameter are reported. The lower boundary of the 97.5% one-sided confidence interval is included within parentheses. CCC_{TOT}, total concordance correlation coefficient considering the intra and inter-observer agreement; CCC_{PREC}, precision sub-index of the concordance correlation coefficient; χ, accuracy; CCC_{INTER}, concordance correlation coefficient for inter-observer agreement; CCC_{INTRA}, concordance correlation coefficient for intra-observer agreement.

TABLE 2 Concordance of each neuropathologist's second reading diagnosis to the consensus classification for histologic grade

Observer	Histologic grade	Well classified samples	Total accuracy
A	Grade I	66/73 (90.4%)	135/156 (86.5%)
	Grade II	58/72 (79.5%)	CI: (80.2%, 91.5%)
	Grade III	11/11 (100.0%)	
B	Grade I	63/73 (86.3%)	141/156 (90.3%)
	Grade II	67/72 (93.1%)	CI: (84.6%, 94.5%)
	Grade III	11/11 (100.0%)	
C	Grade I	43/73 (58.9%)	113/156 (72.4%)
	Grade II	60/72 (83.3%)	CI: (64.7%, 79.3%)
	Grade III	10/11 (90.9%)	
D	Grade I	58/73 (79.5%)	120/156 (76.9%)
	Grade II	53/72 (73.6%)	CI: (69.5%, 83.3%)
	Grade III	9/11 (81.8%)	

Abbreviation: CI, confidence interval.

TABLE 3 Concordance of each neuropathologist's second reading diagnosis to the consensus classification for mitotic grade

Observer	Mitotic grade	Well classified samples	Total accuracy
A	Grade I	79/92 (85.9%)	114/135 (84.4%)
	Grade II	26/34 (76.5%)	CI: (77.2%, 90.1%)
	Grade III	9/9 (100.0%)	
B	Grade I	90/92 (97.8%)	129/135 (95.6%)
	Grade II	30/34 (88.2%)	CI: (90.6%, 98.4%)
	Grade III	9/9 (100.0%)	
C	Grade I	82/92 (89.1%)	111/135 (82.2%)
	Grade II	23/34 (67.6%)	CI: (74.7%, 88.3%)
	Grade III	6/9 (66.7%)	
D	Grade I	83/92 (90.2%)	115/135 (85.2%)
	Grade II	23/34 (67.6%)	CI: (78.1%, 90.7%)
	Grade III	9/11 (88.9%)	

Abbreviation: CI, confidence interval.

agreement was substantial to almost perfect (CCC_{INTRA} ranging from 0.70 to 0.83), while inter-observer agreement was slight to fair (CCC_{INTER} ranging from 0.19 to 0.37) (Table 1).

The most diagnosed histologic subtypes were transitional and meningothelial (50 and 46 cases, respectively) (Table S2). Meningothelial subtype was diagnosed at least by one pathologist in 97 cases out of 186 (52.2%); in 15 samples out of 97 (15.5%) the subtype was unanimous. The transitional subtype was diagnosed at least by one pathologist in 116 out of 186 (62%); in 25 samples out of 116 (21.6%) the subtype was unanimous. In 52 cases, the subtype was not collegially defined.

3.5 | Agreement estimates according to the size of each biological sample

Sample size was available for 185 cases. Of those, 47/185 (25.4%) had an area less than 1 $0mm^2$ (small size) and 138/185 (74.6%) were larger than 10 mm^2 (regular size). For these two groups, the CCC_{TOT}, CCC_{INTRA} and CCC_{INTER} are reported in Table 4. Concerning histologic grade, CCC_{TOT} for small samples was lower than CCC_{TOT} for regular samples (small samples: $CCC_{TOT} = 0.38$; regular sample: $CCC_{TOT} = 0.55$). Regarding intra-observer agreement, CCC_{INTRA} for small and regular samples was similar (small samples: $CCC_{INTRA} = 0.92$; regular sample: $CCC_{INTRA} = 0.87$). Similar results were obtained for mitotic grade. The CCC_{INTER} was lower for small samples than for regular samples (small samples: $CCC_{INTER} = 0.41$; regular samples: $CCC_{INTER} = 0.57$). No substantial differences for CCC_{INTRA} between small and regular samples were observed ($CCC_{INTRA} = 0.83$ and 0.88 respectively).

4 | DISCUSSION

The goal of the present study was to evaluate the reproducibility of criteria used in the human grading when applied to canine meningioma, since it is an essential step before assessing the correlation between grading and prognosis.

Histologic grade. The concordance between individual neuropathologist's histologic grade and the consensus diagnosis ranged from

TABLE 4 Estimates of the concordance correlation coefficients for histologic grade and mitotic grade for small and regular biological samples

	Histologic grade		Mitotic grade	
Index	**Regular**	**Small**	**Regular**	**Small**
CCC_{TOT}	0.55 (0.44)	0.38 (0.25)	0.54 (0.42)	0.37 (0.18)
CCC_{PREC}	0.56 (0.44)	0.41 (0.28)	0.55 (0.44)	0.38 (0.18)
χ	0.99 (0.97)	0.94 (0.87)	0.99 (0.97)	0.99 (0.95)
CCC_{INTER}	0.58 (0.47)	0.41 (0.27)	0.57 (0.45)	0.41 (0.20)
CCC_{PREC}	0.58 (0.48)	0.44 (0.30)	0.59 (0.47)	0.41 (0.21)
χ	0.99 (0.97)	0.94 (0.87)	0.97 (0.94)	0.99 (0.94)
CCC_{INTRA}	0.92 (0.89)	0.87 (0.81)	0.88 (0.83)	0.83 (0.73)

Note: Estimates of the total, inter-observer and intra-observer correlation coefficients and respective sub-indices (precision and accuracy) for each histologic parameter are reported; the lower boundary of the 97.5% one-sided confidence interval is included within parentheses. CCC_{TOT}, total concordance correlation coefficient considering the intra and inter-observer agreement; CCC_{PREC}, precision sub-index of the concordance correlation coefficient; χ, accuracy; CCC_{INTER}, Concordance correlation coefficient for inter-observer agreement, CCC_{INTRA}, Concordance correlation coefficient for intra-observer agreement.

59% to 100%, while the agreement among human neuropathologists for the histologic grade is 87.2%.[17] In contrast, whilst in human meningiomas the agreement is high for each tumour grade, in the present study, an almost perfect concordance was registered only for Grade III tumours. Mitotic grade and anaplasia, criteria on which Grade III is assessed, displayed the highest agreement among observers, explaining the high concordance for histologic Grade III. Grade I and Grade II tumours had lower concordance, suggesting that histologic criteria for grading tumours as I or II (mitotic index, invasion, sheeting architecture, small cells, hypercellularity, macronucleoli and spontaneous necrosis) are more susceptible to subjective interpretation. Considering all the examined cases, low inter-observer agreement was recorded for macronucleoli, small cells, hypercellularity and sheeting architecture. These criteria represent four out of five criteria used to grade tumours as I or II, and they also represent the less agreed criteria among human neuropathologists ($\kappa < 0.5$).[17] Tumour invasion and necrosis displayed moderate agreement. Since tumour invasion is sufficient to classify a meningioma as Grade II, the inter-observer disagreement for invasion could have contributed to the lower concordance for tumour histologic Grades I and II.

Mitotic grade. Mitotic grade is one of the three main criteria (along with invasion and anaplasia) directly linked to histologic grade. In human medicine, mitotic grade is considered a more reproducible criterion, and therefore, up to 75% of meningiomas are graded based on mitotic count.[24] In the present study, inter-observer agreement was moderate, which is comparable to reported inter-observer agreement between human neuropathologists (for mitoses between 4 and 20 in 10 HPF, $\kappa = 0.51$).[17] As described in several studies, mitotic cells are often difficult to differentiate from pyknotic and karyorrhectic cells, infiltrating leukocytes and fixation-related artefacts.[24–26] This difficulty was exacerbated by slide digitisation at ×20 magnification, resulting in a not optimal resolution, as well as by different monitor size, resolution and colour calibration, used by the participants.[26] No recommendations on scanner magnification or monitors have been published yet for diagnostic pathology, though it is common use in diagnostic laboratories to digitise at ×40 and use at least a 28 in. monitor.

Another cause of mitotic grade disagreement in this study was the lack of mitotic count standardisation. Mitoses were counted in three different ways, depending on the observer's interpretation of the criterion: (1) in 10 consecutive fields of 0.237 mm^2 within the most mitotically-active area; (2) in 10 different fields of 0.237 mm^2 dispersed throughout the tumour; (3) in a randomly chosen area of 2.37 mm^2. The most recent WHO human meningioma grading system indicates to count mitoses in consecutive high power fields,[27] in veterinary literature it is preconize to count mitoses in consecutive fields of the most mitotically-active area.[24] Therefore, in order to standardise mitotic count in canine meningioma, we propose that mitoses should be counted in 10 consecutive fields of 0.237 mm^2 within the most mitotically-active area, as recommended by the current literature.[24,28]

The WHO human meningioma classification cut-off of 4 and 20 mitoses is assessed in 1.60 mm^2, consisting of the area obtained by counting 10 fields at ×400 magnification with a microscope field number equaling 18.[27–29] Thus, if mitoses are counted in an area of 2.37 mm^2 in dogs, the cut-offs should be readjusted to 8 and 41. Furthermore, the authors strongly encourage further studies on canine mitotic count cut-off values, since, to the best of our knowledge, there is no evidence that the human cut-offs have a prognostic significance in canine meningioma.

In the present study, inter-observer disagreement between mitotic Grade 1 and 2 was often due to a difference of only 1–3 counted mitoses. An oversight of 1–3 mitoses could be associated with the different amount of time spent by each observer reading slides, as suggested by Rogers,[30] and/or with a different chosen area to count mitoses. To reduce discrepancies, when the mitotic count is 1–2 mitoses lower than the cut-off, mitoses should be re-counted in another highly mitotic area.

Invasion (Figures 1 and 2). Agreement on tumour invasion was fair, and, hence, much lower than in human meningiomas ($\kappa = 0.76$).[30] In human medicine, tumour invasion is correlated to tumour recurrence, therefore invasion is considered sufficient to upgrade a meningioma from Grade I to Grade II.[29,31] Despite the fact that no prognostic studies have been published in dogs, it seems biologically reasonable that invasion could have a similar prognostic impact in dogs. Thus, the presence of brain tissue adjacent to the tumour sample is strongly recommended.

A standardised definition of nervous system invasion is often lacking in the recent veterinary literature.[28] We recommend using the pathology human definition: 'an extension of tumour cells beyond the pial surface into the adjacent brain parenchyma'.[29,32] In most cases, invasion is represented by tongue/finger-like protrusions of the tumour into the central nervous system, while invasion is less

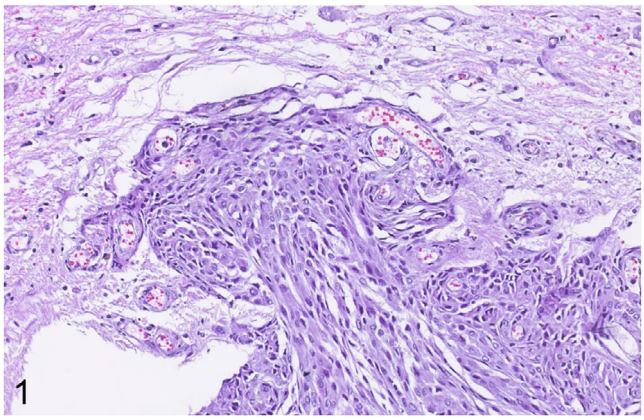

FIGURE 1 Invasive, meningioma, dog, case 43. Tongue-like protrusion of meningioma tumour cells into the brain tissue. Haematoxylin and eosin

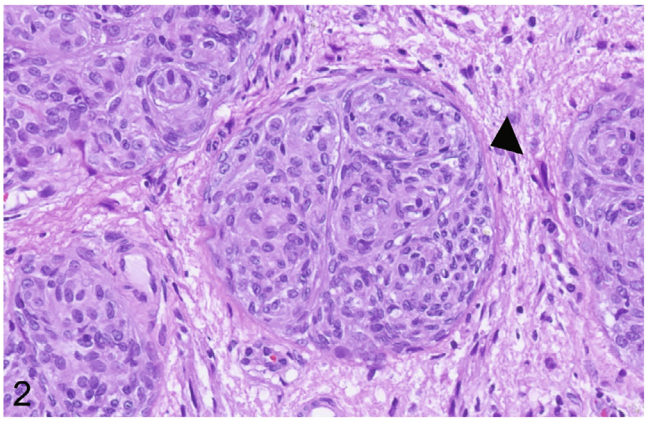

FIGURE 2 Not invasive tumour, meningioma, dog, case 28. Tumour nest, though within brain parenchyma, is surrounded by a visible layer of eosinophilic spindle pial cells (arrowhead). Haematoxylin and eosin

frequently represented by migration of single neoplastic cells or islands of tumour cells into brain tissue.[29,31] Peripheral nervous system invasion, as well as bone and dura infiltration, even if they have an impact on complete surgical resection, are not considered proper tumour invasion of the central nervous system.[29,32] Moreover, tumour cells in Virchow-Robin spaces are not considered an invasion, since this space is lined by pia cells.[27,29,32] Discerning the presence/absence of a layer of fusiform meningeal cells around the tumour could be challenging, especially in dogs where specific canine meningeal markers are lacking. Since tumour invasion into the central nervous system is associated with reactive astrocytosis,[29] in human medicine, GFAP-staining is recommended to assess the presence of an astrocytic reaction on the invasive front.[25,27,31,33] However, the authors think that while helpful, astrocytosis should not be considered a sufficient parameter to assess invasion, since it can be observed in response to other stimuli, like tumour compression and perilesional edema.

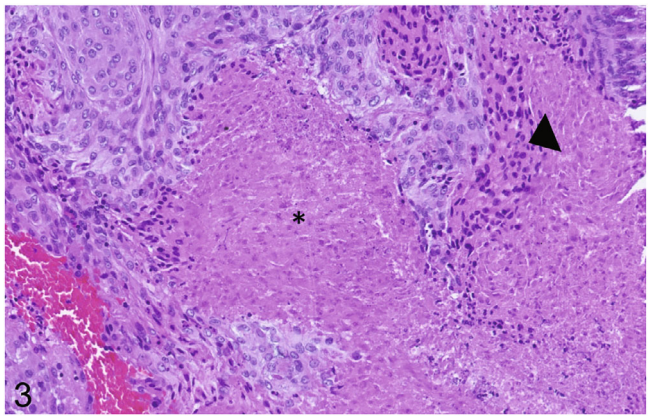

FIGURE 3 Spontaneous necrosis, meningioma, dog, case 72. A large area of spontaneous necrosis (asterisk) is visible. It is composed by a centre of eosinophilic amorphous material, lined by dying cells (arrowhead), that lack cell borders and have a hypereosinophilic cytoplasm and condensed chromatin. Viable tumour cells are present at the periphery. Haematoxylin and eosin

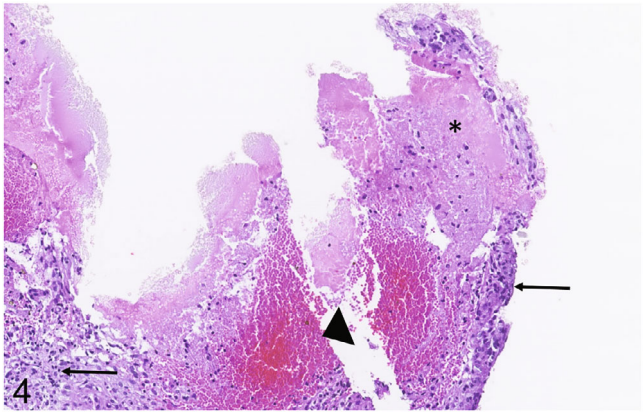

FIGURE 4 Artefactual necrosis, brain, dog, case 23. At the edge of this small sample, measuring approximately 2.4 mm^2, a focal area of necrosis admixed with fibrin is visible (asterisk). It is close to a group of extravasated erythrocytes (arrowhead) in the absence of hemosiderin-laden macrophages, indicating a recent haemorrhage, probably caused by biopsy technique or tissue manipulation before fixation. Few tumour cells are also present (arrows). Haematoxylin and eosin

Spontaneous necrosis (Figure 3). In this study, inter-observer agreement on spontaneous necrosis was moderate, compared to our human counterparts ($\kappa = 0.66$).[17] Although small, rare and/or solitary foci could have been difficult to detect for some participating neuropathologists, the main reason for disagreement was different interpretation of necrotic foci. Small necrotic foci, located at the edge of samples, were reported by some observers as necrosis, while others interpreted these foci as artefacts related to sample handling (Figure 4). Similarly, large haemorrhages were interpreted as necrotic foci by some participants, because of tissue loss due to tissue tearing

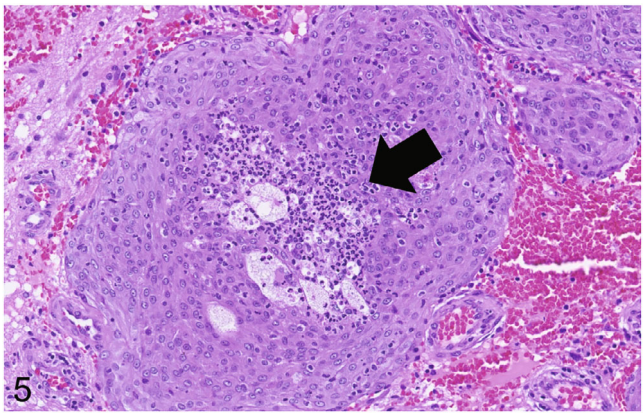

FIGURE 5 Neutrophilic accumulation in necrotic foci, brain, dog, case 72. In the centre of a neoplastic lobule, tumour cells are replaced by small areas of amorphous eosinophilic material, consistent with necrotic material and a moderate number of neutrophils (arrow). Some neutrophils infiltrate the tumour lobule towards the necrotic centre

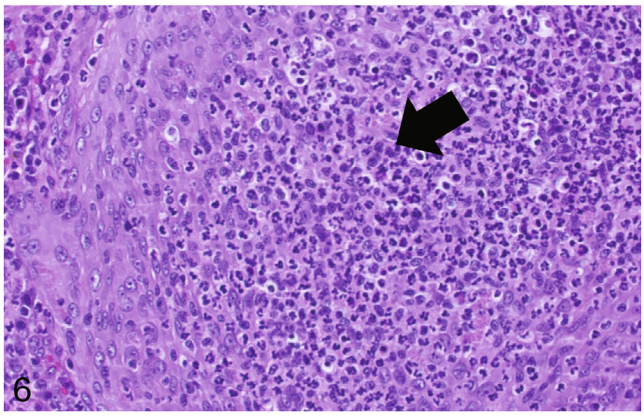

FIGURE 6 Neutrophilic accumulation in necrotic foci, brain, dog, case 72. Tumour is infiltrated by a large amount of neutrophils, forming a microabscess (arrow). Haematoxylin and eosin

and secondary traumatic or ischemic cell necrosis. Furthermore, some participants interpreted intra-tumoral abscesses as necrotic foci, because neutrophils may have induced tumour cell necrosis, or neutrophil chemotaxis could have been triggered by previous spontaneous tumour necrosis (Figures 5 and 6). In order to increase reproducibility in necrosis evaluation, the authors suggest: reporting spontaneous necrosis when present inside the sample; ruling out artefact if necrosis is located at the sample margins or in hemorrhagic areas; considering small and large foci equally; considering abscesses as necrotic foci if cell necrosis is evident inside or around the abscess.

Macronucleoli (Figures 7–10). In the present study, macronucleoli represented one of the soft criteria with the least reproducibility in agreement. During the review process, neuropathologists identified two main reasons for macronucleoli misinterpretation: the exact meaning of macronucleoli is unclear and there are no standardised methods for screening macronucleoli.

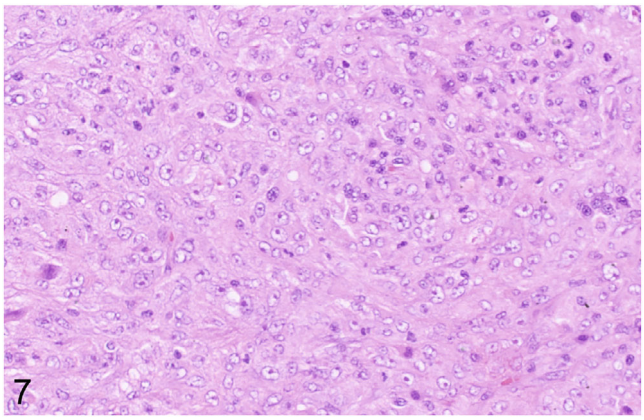

FIGURE 7 Macronucleoli, meningioma, dog, case 161. Neoplastic cell nuclei are vesicular and contain a large eosinophilic nucleolus, evaluated as a macronucleolus

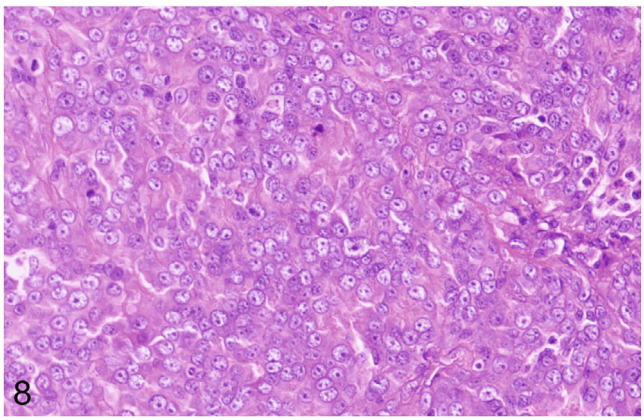

FIGURE 8 Macronucleoli, meningioma, dog, case 108. Neoplastic cell nuclei are vesicular and contain a easily visible nucleolus, evaluated as a macronucleolus

In veterinary literature, canine macronucleoli are neither defined nor measured, leading to a subjective interpretation of this parameter.[11,15] The vast majority of macronucleoli identified in the canine meningiomas in this study were characterised by clumped chromatin or eosinophilic staining with different sizes and shapes. Nucleoli have a vast range in appearance, which contributed to the different interpretations of macronucleolus by the neuropathologists involved in this study. In human meningioma, nucleoli are defined as macronucleoli when they are easily visible at ×100 magnification. For meningioma grading purposes, the criterion is considered positive even when macronucleoli are focally observed (but are present in more than one cell).[32] Although this definition sounds obsolete in the image analysis era, and is reported to have only moderate agreement even among human neuropathologists ($\kappa = 0.49$), macronucleolar identification is easily applicable by light microscopy.[17,25]

For some of the neuropathologists participating in this study, ×100 magnification seemed too low to identify nucleoli, even when prominent; thus, ×200 magnification was suggested. Nevertheless, in

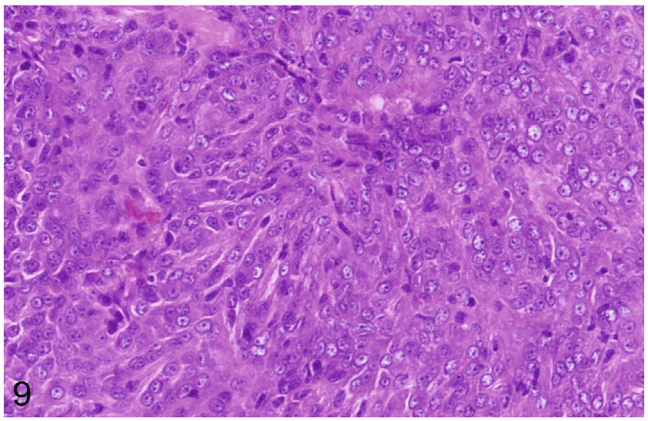

FIGURE 9 Small nucleoli, meningioma, dog, case 5: Tumour cells contain small nucleoli. Chromatin is finely dispersed. Haematoxylin and eosin

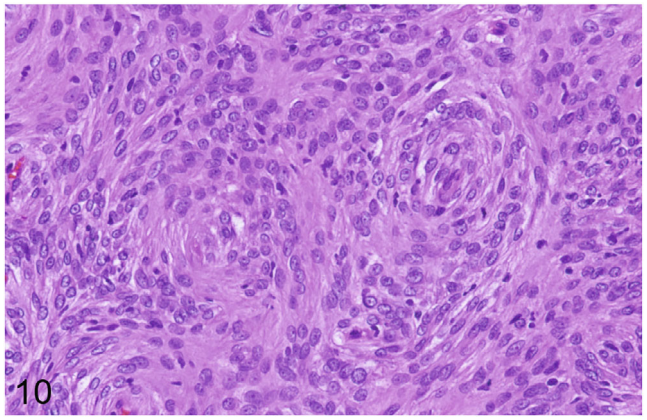

FIGURE 10 Small nucleoli, meningioma, dog, case 6: Tumour cells contain almost not visible nucleoli. Chromatin is finely dispersed. Haematoxylin and eosin

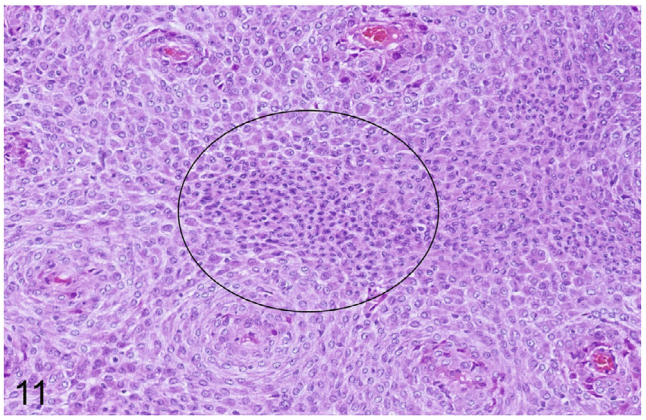

FIGURE 11 Small cells, meningioma, dog, case 49. Among tumour whorls of a meningothelial meningioma, there is a cluster of small cells (encircled), characterised by reduced size and condensed chromatin. Despite the small size, cellularity is not increased

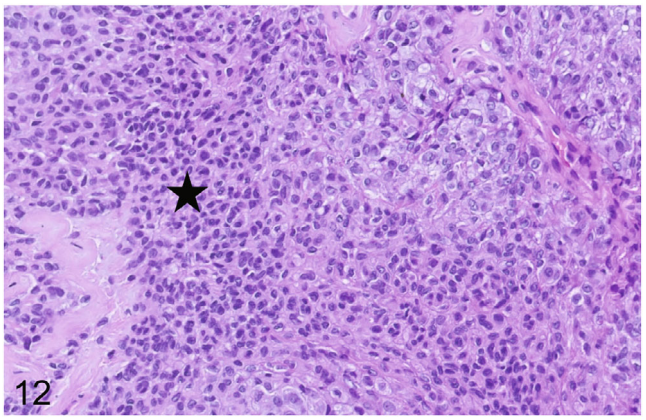

FIGURE 12 Small cells, meningioma, dog, case 69. Most of the tumour cells are small (asterisk), with condensed chromatin mimicking lymphocytes. The high number of small cells in the cluster is also suggestive of foci of hypercellularity. Haematoxylin and eosin

the absence of validated studies, we still recommend using the WHO human definition for macronucleoli identified at ×100 magnification when grading canine meningioma.

Another potential reason for disagreement regarding macronucleoli was the different screen size, ranging from 19 to 27 in., used by observers in this study. Therefore, we recommend adopting a standardised magnification to evaluate macronucleoli, and if working on digitalized slides, we recommend calculating the magnification using the scale bar (not the viewer magnification). Specifically, for a ×100 magnification, the scale bar on the screen should be 100 times longer than the displayed length (e.g., for a correct ×100 magnification, the scale bar indicating 500 μm should measure 5 cm on the screen).

Small cells (Figures 11–13). In the present study, the criterion of small cells showed fair agreement, as in human medicine ($\kappa = 0.39$).[17] For participating veterinary neuropathologists, as for human pathologists,[17,24] it was difficult to interpret the term 'small cells', especially in hypercellular areas. Hypercellularity and small cells often became synonymous causing redundancy of these two evaluated criteria. In the WHO human meningioma classification, small cells are considered as having a high nucleus-cytoplasmic ratio and/or resembling lymphocytes.[11,32] In the present study, where no immunohistochemical stains were performed, the similarity between small cells and lymphocytes could have increased the disagreement, especially for cases with a lymphocytic-rich pattern. Therefore, in routine diagnosis, T and B cell immunolabelling could help to discriminate lymphocytes from tumour cells.

Hypercellularity (Figure 14). Hypercellularity displayed the lowest agreement among all evaluated histologic criteria. This was lower than the agreement reported in human medicine ($\kappa = 0.45$).[17] Several factors could have contributed to this result. First, in the veterinary literature, details defining hypercellularity are lacking, leaving observers to make a subjective interpretation of the criterion. In human medicine, a number of methods have been applied to make the identification of

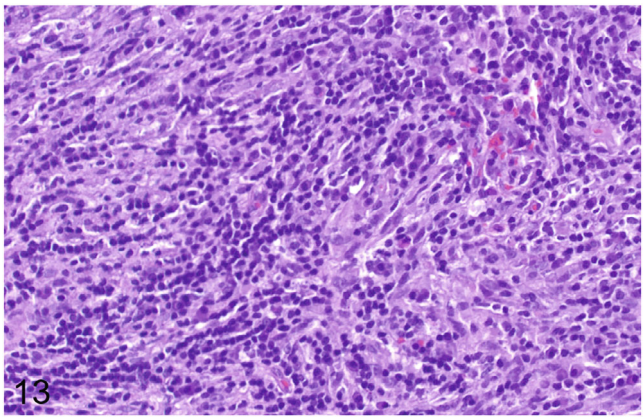

FIGURE 13 Lymphocytic-rich pattern, meningioma, dog, case 97. In this area, a great number of lymphocytes, mimicking tumour small cells, obscures the meningothelial pattern. Haematoxylin and eosin

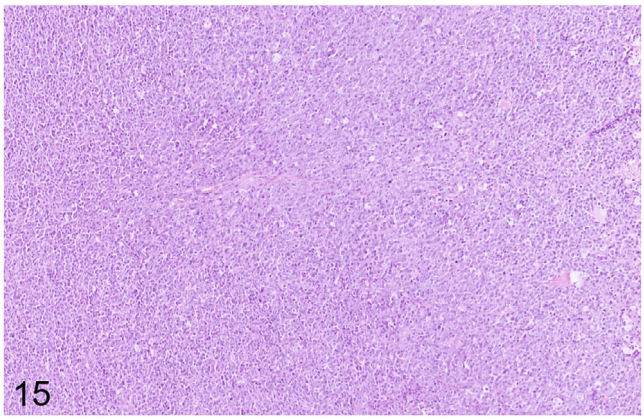

FIGURE 15 Anaplasia and mitoses, meningioma, dog, case 179. In this Grade III meningioma, no specific tumour architecture is recognisable, since cells are growing in sheets

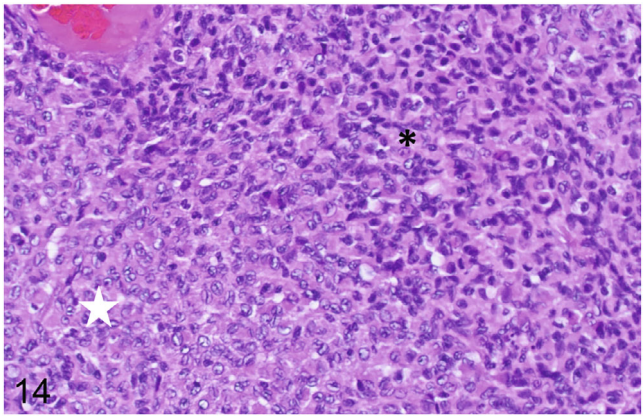

FIGURE 14 Hypercellularity, meningioma, dog, cases 62. An area of basophilic hypercellularity (asterisk) in an otherwise clear eosinophilic tumour (white star). Haematoxylin and eosin

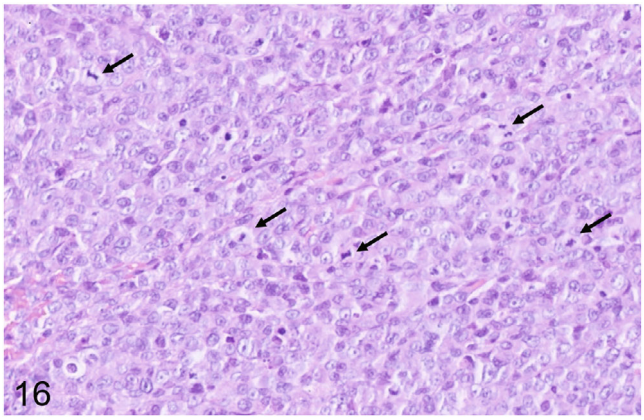

FIGURE 16 Anaplasia and mitoses, meningioma, dog, case 179. A high number of mitotic cells (arrows) are visible. Tumour cells are increased in number (hypercellularity) and anaplastic. Haematoxylin and eosin

hypercellularity less subjective and provide more reproducible results.[24] One method defines increased cellularity as the presence of more than 53 cells in 0.058 mm^2 (corresponding to 217 cells in 0.237 mm^2).[24,32] This cell density seems to be unrealistic in canine meningioma, since in the present study the recorded cell number in 0.237 mm^2 varied between 538 and 3567 (data not shown). Second, in the present study, hypercellularity could have been interpreted in relation to the mean cellularity of that specific tumour subtype and not as an absolute parameter. And finally, in daily routine grading of human meningioma, hypercellularity is semi-quantitatively evaluated by neuropathologists at low magnification.[24,32] Semi-quantitative evaluation of increased cellularity remains subjective,[25] which should be taken into account when a grading system for canine meningioma is correlated to prognosis. In conclusion, though subjective, hypercellularity should be evaluated as an absolute criterion (not subtype-related) at low magnification and should be reported in canine meningioma, even when focally observed.[29,32]

Sheeting architecture (Figures 15 and 16). Sheeting architecture displayed a fair agreement, comparable to the agreement among human pathologists ($\kappa = 0.41$).[17]

Canine meningioma often expresses more than one growth pattern, making it challenging to recognise sheeting, especially at high magnification. For example, the syncytial architecture of meningothelial meningioma can mimic sheeting at high magnification.[17] Moreover, some histologic subtypes, like clear cell, rhabdoid or microcystic, are characterised by cell sheeting,[24,25] but this architecture is subtype-specific and does not indicate dedifferentiation and increased malignancy. For these reasons, in order to clarify the criterion and increase reproducibility in canine meningioma grading, we suggest replacing 'sheeting architecture' with the term 'pattern loss', meaning the lack of a typical meningioma growth pattern.[24] Pattern loss should be evaluated at low magnification and reported when present in more than 50% of the tumour.[24,32]

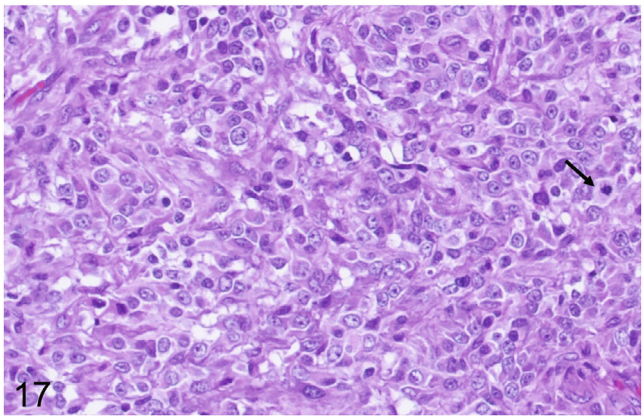

FIGURE 17 Anaplasia, meningioma, dog, case 41. Anaplastic cells, showing different shape and size. The grow pattern is not recognisable, rendering meningioma diagnosis hard to make without special stains. Some mitotic cells are also visible (arrows), indicating a high mitotic grade. Haematoxylin and eosin

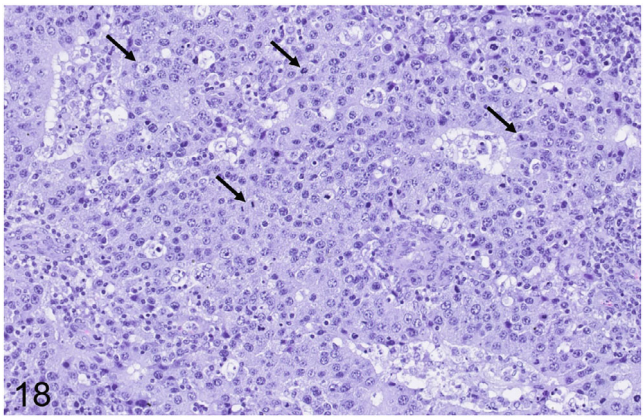

FIGURE 18 Anaplasia, meningioma, dog, case 162. Anaplastic cells, showing different shape and size. The grow pattern is not recognizable, rendering meningioma diagnosis hard to make without special stains. Some mitotic cells are also visible (arrows), indicating a high mitotic grade. Hematoxylin and eosin

TABLE 5 Recommendations of the consortium for reproducible criteria for canine meningioma grading

Histologic criteria	Definition
Mitotic count	Evaluated: • in the most mitotic area • in consecutive high power fields to cover 2.37 mm^2 area • Grade II is defined as tumours with ≥8 mitoses in 2.37 mm^2 • Grade III is defined as tumours with ≥41 mitoses in 2.37 mm^2 • when mitotic count is closely lower than the cut-off (1–2 mitoses of difference), a supplemental count should be performed in another highly mitotic area
Invasion	Presence of tumour cells into the brain or the spinal cord, not surrounded by a pial layer
Necrosis	Focal or multifocal presence of spontaneous necrosis Small and large foci are equally considered When located at sample margins or in hemorrhagic areas, artefactual necrosis should be ruled out In abscesses, reported if tumour cell necrosis is evident
Macronucleoli	Focal or multifocal presence of nucleoli visible at ×100
Small cells	Focal or multifocal presence of cells with a high nucleus/cytoplasmic ratio or with a lymphocytic appearance Evaluated at low magnification
Hypercellularity	Focal or multifocal Evaluated at low magnification Evaluated separately from small cells
Pattern loss	Not identifiable architectural pattern in more than 50% of the tumour surface Evaluated at low magnification
Anaplasia	Multifocal or diffuse presence of anaplastic cells, whose meningeal origin is not evident

Anaplasia (Figures 16–18). Anaplasia displayed a moderate agreement among observers in this study, comparable to agreement among human pathologists ($\kappa = 0.53$).[17] Anaplasia is defined as overtly malignant cytology, rendering it impossible to differentiate meningioma from carcinoma, melanoma or high-grade sarcoma.[29,32] Terms such as 'extreme', taken from veterinary literature,[15,28] should be avoided, because they are misleading and may increase the variability in interpretation of anaplasia intensity. In the present study, anaplasia was observed focally, multifocally or diffusely. In the absence of prognostic studies, the authors concluded that anaplasia, when present focally or affecting small clusters of cells, should not be considered to indicate Grade III, but should be reported to clinicians as a comment; however, when present multifocally or diffusely, anaplasia should be considered sufficient to upgrade the tumour to Grade III.

Sample size. With the spreading of image techniques, in the near future biopsies are going to increase in number, and sample size will have more relevance in diagnostic routines. For human meningioma, six blocks per tumour are recommended to achieve 95% accuracy in grading Grade II tumours.[34] In the present study, reduced size contributed to the decreased inter-observer agreement, since samples less than 10 mm^2 in size obtained only a fair agreement for both histologic and mitotic grade (CCC_{TOT} for histologic grade = 0.38; for mitotic grade = 0.37). Small samples are more easily affected by artefactual changes due to handling, which impedes the assessment of some criteria (necrosis, mitotic figures). Moreover, since most criteria can be expressed multifocally, it is important to evaluate as much tissue as possible.[34]

Tumour subtype. In human medicine, though 15 meningioma subtypes are described, few of them present a real prognostic value. Canine meningiomas display different tumour subtypes, often more than one in the same sample. This heterogeneity makes sometimes difficult to attribute a prevalent subtype to the tumour, increasing the

inter-observer disagreement, as demonstrated in the present study. To date, studies on wide cohorts correlating the subtype to prognosis have not been reported yet.[27,29]

For all the evaluated criteria, intra-observer agreement was substantial to almost perfect showing that the disagreement was almost all due to inter-observer disagreement.[35] As in human neuropathology, a regular discussion on different cases should be encouraged in veterinary pathology, to better define the more subjective features of a disease entity, to homogenously educate pathologists and, ultimately, to increase diagnostic accuracy.[17,24,30]

Robust and reproducible histologic tumour diagnosis and grading is an essential part of providing adequate patient care and treatment. In the present study, a lack of precise definitions for histologic grading criteria for canine meningioma forced pathologists to make subjective interpretations. This was considered the main reason for the observed variation in grading among pathologists. Therefore, the authors issued recommendations to standardise definitions of histologic criteria aimed to improve the reproducibility of canine meningioma grading (Table 5). Unfortunately, in both human and canine meningioma grading, several criteria are still not completely standardised, leading to subjective interpretation by observers.

The use of digitalized slides probably contributed to the low inter-observer agreement, because of the lack of validated process, the lack of users training on digital pathology and the lack of standardised workstation.[35] It is out of the scope of the present manuscript to give recommendations on digital pathology, but readers are strongly encouraged to read the most recent specialised literature on the topic.

Although our results and recommendations can contribute to standardise the grading system for canine meningioma, further work is needed to generate a reproducible grading system for canine meningioma that is correlated to prognosis.

ACKNOWLEDGEMENTS

The authors thank all the technicians and pathologists involved in their daily work and Dr. Leah Cannon for editing support. Without their excellent work, studies like this one would never be completed.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Snyder JM, Shofer FS, Van Winkle TJ, Massicotte C. Canine intracranial primary neoplasia: 173 cases (1986-2003). *J Vet Intern Med*. 2006;20(3):669-675.
2. Song RB, Vite CH, Bradley CW, Cross JR. Postmortem evaluation of 435 cases of intracranial neoplasia in dogs and relationship of neoplasm with breed, age, and body weight. *J Vet Intern Med*. 2013;27(5):1143-1152. doi:10.1111/jvim.12136
3. Foster ES, Carrillo JM, Patnaik AK. Clinical signs of tumors affecting the rostral cerebrum in 43 dogs. *J Vet Intern Med*. 1988;2(2):71-74. doi:10.1111/j.1939-1676.1988.tb02796.x
4. Petersen SA, Sturges BK, Dickinson PJ, et al. Canine intraspinal meningiomas: imaging features, histopathologic classification, and long-term outcome in 34 dogs. *J Vet Intern Med*. 2008;22(4):946-953. doi:10.1111/j.1939-1676.2008.0106.x
5. Schwartz M, Lamb CR, Brodbelt DC, Volk HA. Canine intracranial neoplasia: clinical risk factors for development of epileptic seizures. *J Small Anim Pract*. 2011;52(12):632-637. doi:10.1111/j.1748-5827.2011.01131.x
6. Lacassagne K, Hearon K, Berg J, et al. Canine spinal meningiomas and nerve sheath tumours in 34 dogs (2008-2016): distribution and long-term outcome based upon histopathology and treatment modality. *Vet Comp Oncol*. 2018;16(3):344-351. doi:10.1111/vco.12385
7. Platt SR, Scase TJ, Adams V, et al. Vascular endothelial growth factor expression in canine intracranial meningiomas and association with patient survival. *J Vet Intern Med*. 2006;20(3):663-668. doi:10.1892/0891-6640(2006)20[663:vegfei]2.0.co;2
8. Suñol A, Mascort J, Font C, Bastante AR, Pumarola M, Feliu-Pascual AL. Long-term follow-up of surgical resection alone for primary intracranial rostrotentorial tumors in dogs: 29 cases (2002-2013). *Open Vet J*. 2017;7(4):375-383. doi:10.4314/ovj.v7i4.14
9. Motta L, Mandara MT, Skerritt GC. Canine and feline intracranial meningiomas: an updated review. *Vet J Lond Engl*. 2012;192(2):153-165. doi:10.1016/j.tvjl.2011.10.008
10. Koestner A. *Histological Classification of Tumors of the Nervous System of Domestic Animals*; Armed Force Institute of Pathology; 1999.
11. Higgins RJ, Bollen AW, Dickinsons PJ, Siso-Llonch S. Tumors of the nervous system. In: Meuten DJ, ed. *Tumors in Domestic Animals*. Vol 1. John Wiley & Sons, Inc.; 2017:834-891.
12. Mandara MT, Pavone S, Mandrioli L, Bettini G, Falzone C, Baroni M. Matrix metalloproteinase-2 and matrix metalloproteinase-9 expression in canine and feline meningioma. *Vet Pathol*. 2009;46(5):836-845. doi:10.1354/vp.08-VP-0185-M-FL
13. Miller AD, Miller CR, Rossmeisl JH. Canine primary intracranial cancer: a Clinicopathologic and comparative review of glioma, meningioma, and choroid plexus tumors. *Front Oncol*. 2019;9:1151. doi:10.3389/fonc.2019.01151
14. Sturges BK, Dickinson PJ, Bollen AW, et al. Magnetic resonance imaging and histological classification of intracranial meningiomas in 112 dogs. *J Vet Intern Med*. 2008;22(3):586-595. doi:10.1111/j.1939-1676.2008.00042.x
15. Vandevelde M, Higgins RJ, Oevermann A. Neoplasia. *Veterinary Neuropathology: Essentials of Theory and Practice*. John Wiley & Sons, Inc; 2012.
16. Rotondi MA, Donner A. A confidence interval approach to sample size estimation for interobserver agreement studies with multiple raters and outcomes. *J Clin Epidemiol*. 2012;65(7):778-784. doi:10.1016/j.jclinepi.2011.10.019
17. Rogers CL, Perry A, Pugh S, et al. Pathology concordance levels for meningioma classification and grading in NRG oncology RTOG trial 0539. *Neuro-Oncology*. 2016;18(4):565-574.
18. R Foundation for Statistical, Computing, Vienna, Austria. R: The R Project for Statistical Computing. Accessed June 10, 2021. https://www.r-project.org/
19. Lin L, Hedayat AS, Wu W. *Statistical Tools for Measuring Agreement*. Springer-Verlag; 2012. doi:10.1007/978-1-4614-0562-7

20. Gwet KL. irrCAC: computing chance-corrected agreement coefficients (CAC). Accessed June 10, 2021. https://CRAN.R-project.org/package=irrCAC
21. Berthold MR, Cebron N, Dill F, et al. KNIME: the Konstanz information miner. In: Preisach C, Burkhardt H, Schmidt-Thieme L, Decker R, eds. *Data Analysis, Machine Learning and Applications. Studies in Classification, Data Analysis, and Knowledge Organization*. Springer; 2008: 319-326. doi:10.1007/978-3-540-78246-9_38
22. Preisach C, Burkhardt H, Schmidt-Thieme B, Decker R, eds. *Data Analysis, Machine Learning and Applications: Proceedings of the 31st Annual Conference of the Gesellschaft Für Klassifikation e.V., Albert-Ludwigs-Universität Freiburg, March 7–9, 2007*. Springer-Verlag; 2008. doi:10.1007/978-3-540-78246-9
23. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics*. 1977;33(1):159-174. doi:10.2307/2529310
24. Backer-Grøndahl T, Moen BH, Torp SH. The histopathological spectrum of human meningiomas. *Int J Clin Exp Pathol*. 2012;5(3):231-242.
25. Barresi V, Caffo M, Tuccari G. Classification of human meningiomas: lights, shadows, and future perspectives. *J Neurosci Res*. 2016;94(12):1604-1612. doi:10.1002/jnr.23801
26. Donovan TA, Moore FM, Bertram CA, et al. Mitotic figures-normal, atypical, and imposters: a guide to identification. *Vet Pathol*. 2021;58(2):243-257. doi:10.1177/0300985820980049
27. Sahm F, Perry A, von Deimling A, et al. Meningioma. *WHO Classification of Tumours Editorial Board. Central Nervous System Tumours [Internet]*. Vol 6. 5th ed. International Agnecy for Research in Cancer; 2021. https://tumourclassification.iarc.who.int/chapters/45
28. Meuten DJ, Moore FM, George JW. Mitotic count and the field of view area: time to standardize. *Vet Pathol*. 2016;53(1):7-9. doi:10.1177/0300985815593349
29. Louis DN, Ohgaki H, Wiestler OD, et al. In: Jaffe ES, Lakhani SR, Ohgaky H, eds. *WHO Classification of Tumours of the Central Nervous System*. Vol 1. International Agency for Research on Cancer (IARC); 2016.
30. Rogers L, Gilbert M, Vogelbaum MA. Intracranial meningiomas of atypical (WHO grade II) histology. *J Neurooncol*. 2010;99(3):393-405. doi:10.1007/s11060-010-0343-1
31. Brokinkel B, Hess K, Mawrin C. Brain invasion in meningiomas—clinical considerations and impact of neuropathological evaluation: a systematic review. *Neuro-Oncology*. 2017;19(10):1298-1307. doi:10.1093/neuonc/nox071
32. Arie Perry DJB. *Practical Surgical Neuropathology a Diagnostic Approach*. Vol 1. 2nd ed. Elsevier; 2018.
33. Banan R, Abbetmeier-Basse M, Hong B, et al. The prognostic significance of clinicopathological features in meningiomas: microscopic brain invasion can predict patient outcome in otherwise benign meningiomas. *Neuropathol Appl Neurobiol*. 2021;47:724-735. doi:10.1111/nan.12700
34. Dal Col P, Garaix T, Massard A, et al. Meningioma sampling: how much is enough for the accurate grading of atypical meningiomas? *Pathology*. 2021;53:602-607. doi:10.1016/j.pathol.2020.10.024
35. Bertram CA, Stathonikos N, Donovan TA, et al. Validation of digital microscopy: review of validation methods and sources of bias. *Vet Pathol*. 2022;59(1):26-38. doi:10.1177/03009858211040476

SUPPORTING INFORMATION

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How to cite this article: Belluco S, Marano G, Baiker K, et al. Standardisation of canine meningioma grading: Inter-observer agreement and recommendations for reproducible histopathologic criteria. *Vet Comp Oncol*. 2022;20(2):509-520. doi:10.1111/vco.12802