Received: 27 December 2022 | Revised: 7 August 2023 | Accepted: 10 August 2023

DOI: 10.1111/vco.12932

ORIGINAL ARTICLE

Veterinary and Comparative Oncology WILEY

Standardization of canine meningioma grading: Validation of new guidelines for reproducible histopathologic criteria

Sara Belluco[1] | Giuseppe Marano[2] | Thibaut Lurier[3,4] | Giancarlo Avallone[5] | Chiara Brachelente[6] | Stefano Di Palma[7] | Roberta Rasotto[8] | Kerstin Baiker[9] | Andreas Beineke[10] | Anna Oevermann[11] | Frauke Seehusen[12] | Fabiano José Ferreira de Sant'Ana[13] | Patrizia Boracchi[2] | Martí Pumarola[14] | Maria Teresa Mandara[15]

[1]Université de Lyon, VetAgro Sup, ICE UPSP 2016.A104, Axe Cancérologie, Marcy l'Etoile, Lyon, France

[2]Department of Biomedical and Clinical Sciences "L. Sacco", University of Milan, Milan, Italy

[3]INRAE, VetAgro Sup, UMR EPIA, Université Clermont Auvergne, Saint-Genès-Champanelle, France

[4]INRAE, VetAgro Sup, UMR EPIA, Université de Lyon, Lyon, France

[5]Dipartimento di Scienze Mediche Veterinarie, Università di Bologna, Bologna, Italy

[6]Dipartimento di Medicina Veterinaria, Università degli Studi di Perugia, Perugia, Italy

[7]IDEXX Laboratories, Wetherby, UK

[8]Diagnostic Pathology, Dick White Referrals, Station Farm, Cambridgeshire, UK

[9]Department of Veterinary Clinical Sciences, Jockey Club College of Veterinary Medicine and Science, City University of Hong Kong, Kowloon, Hong Kong

[10]Stiftung Tierärztliche Hochschule Hannover, Institut für Pathologie, Hannover, Germany

[11]Division of Neurological Sciences, Vetsuisse Faculty, University of Bern, Bern, Switzerland

[12]Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

[13]Laboratório de Diagnóstico Patológico Veterinário, Universidade de Brasília, Brasília, Brazil

[14]Dept Medicina i Cirurgia, Animals, Facultat de Veterinària, Barcelona, Spain

[15]Laboratorio di Neuropatologia, Dip. di Medicina Veterinaria, Università degli Studi di Perugia, Perugia, Italy

Correspondence
Sara Belluco, Laboratoire d'histopathologie vétérinaire, VetAgro Sup, Campus vétérinaire, 1, avenue Bourgelat, 69280 Marcy l'étoile, France.
Email: sara.belluco@vetagro-sup.fr

Funding information
VetAgro Sup

Abstract

Canine meningiomas are currently graded using the human grading system. Recently published guidelines have adapted the human grading system for use in dogs. The goal of this study was to validate the new guidelines for canine meningiomas. To evaluate the inter-observer agreement, 5 veterinary surgical pathologists graded 158 canine meningiomas following the human grading system alone or with the new guidelines. The inter-observer agreement for histologic grade and each of the grading criteria (mitotic grade, invasion, spontaneous necrosis, macronucleoli, small cells, hypercellularity, pattern loss and anaplasia) was evaluated using the Fleiss kappa index. The diagnostic accuracy (sensitivity and specificity) was assessed by comparing the diagnoses obtained with the 2 grading systems with a consensus grade (considered the reference classification). The consensus histologic grade was obtained by

agreement between 4 experienced veterinary neuropathologists following the guidelines. Compared with the human grading alone, the canine-specific guidelines increased the inter-observer agreement for: histologic grade ($\kappa = 0.52$); invasion ($\kappa = 0.67$); necrosis ($\kappa = 0.62$); small cells ($\kappa = 0.36$); pattern loss ($\kappa = 0.49$) and anaplasia ($\kappa = 0.55$). Mitotic grade agreement remained substantial ($\kappa = 0.63$). The guidelines improved the sensitivity in identifying grade 1 (95.6%) and the specificity in identifying grade 2 (96.2%) meningiomas. In conclusion, the new grading guidelines for canine meningiomas are associated with an overall improvement in the inter-observer agreement and higher diagnostic accuracy in diagnosing grade 1 and grade 2 meningiomas.

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

1 | INTRODUCTION

Canine meningioma is the most frequent primary central nervous system tumour in dogs, accounting for up to 51% of intra-cranial tumours.[1] Intra-cranial meningioma frequently causes seizures, altered mentation and vestibular syndrome, while intra-spinal meningioma causes ataxia and paresis. One-year survival after surgery is approximately 50%.[2,3] To date, neither clinical factors nor histologic features have been correlated with prognosis in canine meningioma patients. Therefore, the biologic behaviour of the tumour and the patient outcomes cannot be predicted in a clinical setting, resulting in a lack of targeted treatments for affected dogs.

In veterinary medicine, classification of nervous system tumours dates back to 1999.[4] However, this older classification system has several limitations compared with more recent literature. Therefore, based on clinical, imaging and histologic similarities between canine and human meningiomas, histologic grading of canine meningioma is currently performed by applying the histologic criteria in the 2016 WHO human grading system.[5–8] In human medicine, histologic grade is a robust predictor of survival and recurrence in meningioma patients.[9–11] However, the human grading system has not been correlated with prognosis in dogs.

A reproducible and reliable grading system is necessary to correlate histologic findings with prognosis in both human medicine[12–17] and veterinary medicine.[18–21] The reproducibility of the human meningioma grading system applied to canine meningioma was previously investigated to lay the groundwork for studies on the prognostic significance of histologic grade in canine meningiomas. Use of the human grading system to grade canine meningiomas resulted in low reproducibility, possibly due to unclear descriptions of some of the diagnostic criteria in the veterinary literature.[5,6] Therefore new guidelines were recently published to increase the reproducibility of histologic grading applied to canine meningioma.[22]

The goal of this study was to validate the recent guidelines for the assessment of histologic grade in canine meningioma. First, we evaluated the inter-observer agreement among surgical pathologists using the human grading system alone or in combination with the recently published guidelines. Second, we compared the diagnostic accuracy of the two grading systems, performed by surgical pathologists, with a consensus grade obtained by agreement between four experienced veterinary neuropathologists.

2 | METHODS

The research protocol was reviewed and approved by our institutional Research Ethics Committee.

2.1 | Cases

A total of 158 canine meningiomas, previously used to develop the guidelines, were included in this study.[22] The four neuropathologists reached a consensus on histologic and mitotic grades for 151 and 148 cases, respectively.

Tumours were surgically resected for the benefit of the animal and fixed in 10% buffered formalin. For each case, samples were routinely processed for histopathology, cut into 4-μm sections, stained with haematoxylin and eosin and digitized with a NDP scanner (NDP scan 2.5.90, Nanozoomer HT, Hamamatsu) at 20× magnification (454 nm/pixel). Slides were visualized with the freely available NDP.2 viewer (NDP.view2 Viewing software U12388-01, Hamamatsu Photonics).

2.2 | Histologic evaluation of the tumours

Each of the 158 slides was analysed twice by 5 board-certified veterinary surgical pathologists to define the mitotic grade and the histologic grade. The first reading was performed following the human grading system applied to canine meningioma, as previously published in the veterinary literature (Table 1).[5,6] The second reading was done

TABLE 1 Human histologic grading for canine meningioma.[5,6]

Grade	Criteria
Grade 1	Tumours lacking criteria for grades 2 and 3
Grade 2	Tumours with mitotic grade of 2 or tumours with central nervous tissue invasion or tumours displaying at least 3 of the following criteria: • Sheeting architecture • Small cells • Hypercellularity • Macronucleoli • Spontaneous necrosis
Grade 3	Tumours with mitotic grade of 3 or tumours with extreme anaplasia

blindly between 18 and 21 months after the first reading; tumours were graded following the recently published guidelines (Table 2).[22]

Since canine meningioma subtypes have not been definitively correlated with tumour behaviour and prognosis, tumour subtype was not considered for the histologic grade.

2.3 | Mitotic grade

The mitotic grade was calculated by counting the number of detected mitoses according to the human grading system (first reading) or following the new guidelines (second reading). Since the cut-offs for mitotic grade are different in the human grading system and in the guidelines, the mitotic grade was calculated based on the two different cut-offs, as reported in Table 3, and the histologic grade was subsequently calculated.

Inter-observer agreement was calculated for all four mitotic grades obtained.

2.4 | Macronucleoli

In the article outlining the new meningioma guidelines, concern was raised that the 100× magnification might be too low for assessing macronucleoli in canine meningiomas (comment in the discussion[22]). Therefore, in order to evaluate how magnification could influence inter-observer agreement on this criterion and, as a consequence, on the histologic grade, macronucleoli were evaluated at 100× and 200× (adjusted to screen size[22]). Inter-observer agreement was statistically analysed for macronucleoli and histologic grade at 100× and 200×.

2.5 | Statistical analysis

Data consisted of 1580 plus 299 records. The 1580 records included results of histopathologic evaluation of the 158 slides performed by 5 surgical pathologists in 2 readings. The 299 records comprised the consensus evaluations of histologic grade and mitotic grade (151 and 148 records, respectively) performed by neuropathologists. For each of the 1580 evaluations, we recorded the histologic grade (grade 1, 2 and 3) and the following histologic criteria: mitotic grade (grade 1, 2 and 3) evaluated using the old and new cut-off values; spontaneous necrosis (yes/no); macronucleoli (yes/no); small cells (yes/no); increased cellularity (yes/no); pattern loss (yes/no); invasion (yes/no) and anaplasia (yes/no).

TABLE 2 Guidelines for reproducible criteria on canine meningioma grading.[22]

Histologic criteria	Guidelines
Mitotic count	Evaluated: • in the most mitotic area • in consecutive high power fields to cover 2.37 mm^2 area 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 pial layer cells
Spontaneous 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 100X
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	Replace the "sheeting architecture" Defined as not identifiable architectural pattern in more than 50% of the tumour surface Evaluated at low magnification
Anaplasia	Replace "extreme anaplasia" Defined as the multifocal or diffuse presence of anaplastic cells, whose meningeal origin is not evident

TABLE 3 Mitotic grades, following what reported in the human literature as published in veterinary medicine (old cut-off)[5,6] and following the guidelines (new cut-off).[22]

Grade	Old cut-off	New cut-off
1	<4 mitoses in 2.37 mm^2	<8 mitoses in 2.37 mm^2
2	Between 4 and 19 in 2.37 mm^2	Between 8 and 40 in 2.37 mm^2
3	≥20 mitoses in 2.37 mm^2	≥41 mitoses in 2.37 mm^2

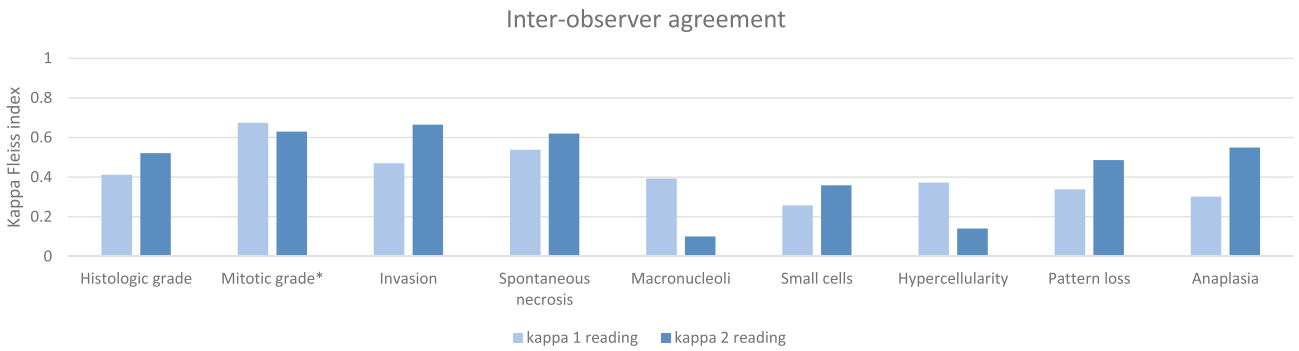

FIGURE 1 Inter-observer agreement among surgical pathologists, following the human grading system alone (first reading) and following the guidelines (second reading). With the guidelines, the inter-observer agreement was increased for histologic grade and five criteria (invasion, spontaneous necrosis, small cells, pattern loss and anaplasia). Agreement for mitotic grade was similar regardless of whether the new guidelines were applied. Agreement decreased when applying the guidelines for two criteria (prominent nucleoli and increased cellularity). *Mitotic grade calculated with the cut-off recommended in the guidelines (new cut-off).

Inter-observer agreement among surgical pathologists using the human grading system (first reading) and the human grading system in combination with the guidelines (second reading) was assessed by estimates of the Fleiss kappa index. The level of agreement was interpreted using the Landis and Koch interpretation[23]: slight agreement for kappa values between 0.00 and 0.20; fair agreement for values between 0.21 and 0.40; moderate agreement for values between 0.41 and 0.60; substantial agreement for values between 0.61 and 0.80; and almost perfect agreement for values between 0.81 and 1.00. Along with the kappa index, the "uncorrected" (not corrected for chance-expected agreement) percentage of agreement was reported, in order to provide additional insights. This index is defined as the percentage of concordant pairs over the total number of paired results for every pair of surgical pathologists.

The methods described above were also used to evaluate the inter-observer agreement for macronucleoli determined at 100× and 200× magnification.

The agreement with the consensus histologic grade (considered as the reference classification) was evaluated by estimates of classification accuracy. In order to account for the association among the classifications performed on each single slide ("within-slide association"), estimates of sensitivity, specificity and respective 95% confidence intervals were obtained by generalized estimating equation (GEE) methods, as described by Genders et al.[24]

Furthermore, differences in sensitivity and specificity between histologic grades evaluated by the human grading alone and using the guidelines were obtained as follows: for each histologic grade (1–3, and), a test of hypothesis on the coefficients of the pertinent GEE model was performed. The null hypothesis of the test was that both sensitivity and specificity are equal between the two methods. In cases of rejection of the null hypothesis ($p < .05$), further results were reported, that is, 95% confidence intervals for the differences of sensitivity and specificity. These confidence intervals were obtained by the non-parametric bootstrap method, with 3000 bootstrap samples.

The analyses were performed using the R software version 4.2.4 (R Core Team,[25] with the packages irrCAC[26] and geepack[27] added and Knime Analytics Platform release 4.6.0.

3 | RESULTS

3.1 | Inter-observer agreement

The inter-observer agreement among surgical pathologists was greater for histologic grade and 5 out of 8 histologic criteria, when the guidelines were applied than when using the human grading system alone (Figure 1 and Table 4). At the first reading (human system alone), three of the nine criteria (histologic grade, invasion and spontaneous necrosis) had moderate agreement, 1 (mitotic grade) had substantial agreement and none had almost perfect agreement. At the second reading (using the guidelines), three of nine criteria had moderate agreement (histologic grade, pattern loss and anaplasia) with increased kappa index and three criteria had substantial agreement (mitotic grade, invasion and spontaneous necrosis). Small cells still had fair agreement, although the kappa index was increased at the second reading, compared with the first reading.

In contrast, macronucleoli and hypercellularity had decreased agreement with the guidelines, compared with the human grading alone, since the agreement went from fair, at the first reading, to slight, at the second reading.

3.2 | Mitotic grade inter-observer agreement

For each reading (first and second), the mitotic grade was evaluated using 2 different cut-offs: the old cut-off, based on the human WHO system and as previously published in the veterinary literature, and the new cut-off suggested by the guidelines. Although differences were not statistically significant (with overlaps between 95% confidence intervals), all inter-observer agreements for mitotic grade were

TABLE 4 Inter observer agreement among surgical pathologists using the human grading as reported in veterinary literature (first reading) and using the guidelines (second reading).

Reading		Histologic grade	Mitotic grade[a]	Invasion	Spontaneous necrosis	Macronucleoli	Small cells	Increased cellularity	Pattern loss	Anaplasia
1st	%	70.6%	94.1%	76.9%	77.7%	72.8%	79.0%	77.0%	71.4%	92.7%
	kappa	0.412	0.674	0.470	0.538	0.393	0.258	0.372	0.339	0.302
	(95% C.I.)	(0.340, 0.484)	(0.550–0.798)	(0.382, 0.558)	(0.457, 0.619)	(0.307, 0.479)	(0.143, 0.374)	(0.271, 0.473)	(0.258, 0.420)	(0.172, 0.432)
2nd	%	77.5%	94.1%	85.2%	81.5%	97.1%	78.0%	63.2%	86.6%	91.5%
	kappa	0.521	0.630	0.665	0.62	0.101	0.359	0.141	0.486	0.549
	(95% C.I.)	(0.436, 0.606)	(0.519–0.742)	(0.584, 0.745)	(0.540, 0.701)	(0.017, 0.184)	(0.265, 0.452)	(0.068, 0.214)	(0.359, 0.614)	(0.393, 0.705)

Note: %: percentage of "concordant" evaluations by two pathologists; kappa = agreement index (Fleiss Kappa).
Abbreviation: C.I., confidence interval.
[a]Mitotic grade evaluated following the new cut-offs proposed in the guidelines.

FIGURE 2 Inter-observer mitotic grade agreement among surgical pathologists, following the human grading system alone (first reading) and following the guidelines (second reading), when 2 different mitotic cut-offs were applied. The cut-off proposed in the guidelines (new) increased the inter-observer agreement, independent of whether or not the guidelines were followed. Nevertheless, there was a slightly decreased agreement among pathologists when the guidelines were followed (second reading), compared to that obtained using the human system alone (first reading), independent of which cut-off was used.

greater and passed from moderate to substantial using the new cut-off, compared with the old one, at both the first and second readings (Figure 2 and Table 5).

Counting mitoses following the guidelines (second reading), slightly lowered the agreement for mitotic grade calculated with both cut-offs, when compared with the first reading. Nevertheless, the overall agreement for histologic grade increased when the guidelines were used (Figure 3 and Table 5).

3.3 | Macronucleoli inter-observer agreement

At 100X magnification, the kappa index was slight, while at 200× magnification, the kappa index was fair. Despite this difference, agreement on the histologic grade only marginally increased when nucleoli were evaluated at 200×, compared with 100× magnification (Figure 4 and Table 6).

3.4 | Concordance with the consensus diagnosis

The 4 veterinary neuropathologists reached a consensus diagnosis for mitotic grade in 148/158 (93.6%) cases by applying the guidelines and the new mitotic grade cut-offs. Among the 148 tumours, there were 134 (90.6%) grade 1 tumours, 11 (7.4%) grade 2 tumours and 3 (2%) grade 3 tumours. Since there were few grade 2 and 3 tumours, diagnostic accuracy for mitotic grade with the guidelines could not be calculated.

Readings	Cut-offs	Agreement	Mitotic grade	Histologic grade
1st reading	Old	%	81.5%	71.8%
		Kappa	0.507	0.458
		95% C.I.	0.407, 0.606	0.379, 0.537
1st reading	New	%	94.1%	70.6%
		Kappa	0.674	0.412
		95% C.I.	0.550, 0.798	0.340, 0.484
2nd reading	Old	%	78.2%	71.9%
		Kappa	0.443	0.485
		95% C.I.	0.350, 0.535	0.406, 0.564
2nd reading	New	%	94.1%	77.5%
		Kappa	0.630	0.521
		95% C.I.	0.519, 0.742	0.436, 0.606

TABLE 5 Inter-observer agreement among surgical pathologists for mitotic and histologic grades, evaluated with the human grading system (first reading), or with the grading system implemented with the guidelines (second reading), applying the two different two cut-offs: the old cut-off, as published in the previous veterinary literature, and the new cut-off, as proposed in guidelines.

Note: %: percentage of "concordant" evaluations by two surgical pathologists.
Abbreviations: C.I., confidence interval; kappa, Fleiss Kappa index.

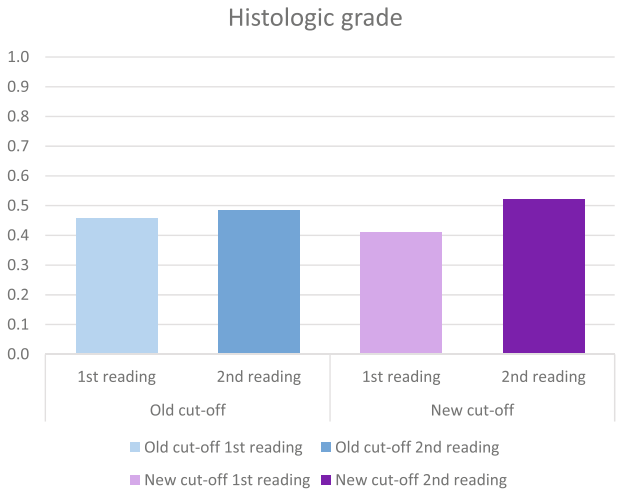

FIGURE 3 Inter-observer histologic grade agreement among surgical pathologists, following the human grading system alone (first reading) and following the guidelines (second reading), when 2 different mitotic cut-offs were applied. With the guidelines (second reading), the inter-observer agreement was increased, independently of which cut-off was used.

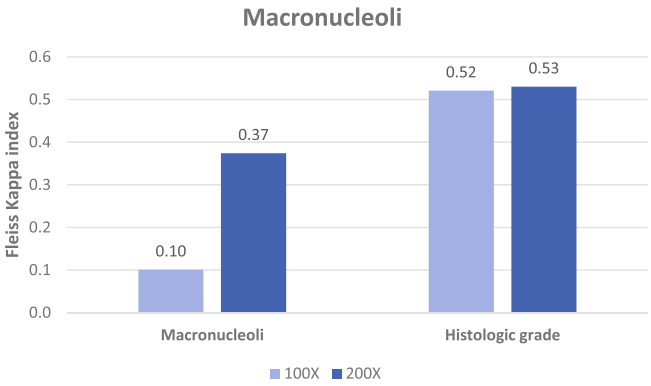

FIGURE 4 Inter-observer agreement among surgical pathologists, for histologic grade and macronucleoli, when macronucleoli were evaluated at 100× and 200× magnification. At 200× magnification, the inter-observer agreement for macronucleoli was increased. However, regardless of which magnification was used, the inter-observer agreement for histologic grade did not change.

The 4 veterinary neuropathologists reached a consensus diagnosis for histologic grade in 151/158 (95.6%) cases by applying the guidelines and the new mitotic grade cut-offs. Among the 151 tumours, there were 78 (51.6%) grade 1 tumours, 62 (41.1%) grade 2 tumours and 11 (7.3%) grade 3 tumours.

The estimates of sensitivities and specificities obtained by the GEE method are reported in Table 7. We found a statistically significant difference between the old grading system and the new guidelines for grade 1 ($p = 0.014$) and grade 2 ($p = 0.016$) tumours. For grade 1 tumours, the sensitivity was increased at 95.6% (+ 3.9%, CI: 0.9%–7.1%) when using the guidelines and this difference was statistically significant. The specificity was decreased (−3.6%, CI −9.1% to 2.1%), but this difference was not statistically significant.

TABLE 6 Inter- observer agreement among surgical pathologists for macronucleoli, evaluated at 100× and 200× magnification.

Magnification	Agreement	Macronucleoli	Histologic grade
100×	%	97.1%	77.5%
	Kappa	0.101	0.521
	(95% C.I.)	0.017, 0.184	0.436, 0.606
200×	%	77.5%	77.3%
	Kappa	0.374	0.530
	(95% C.I.)	0.281, 0.467	0.446, 0.614

Note: % = percentage of concordant evaluations by two surgical pathologists.
Abbreviation: C.I., confidence interval; kappa, agreement index (Fleiss Kappa).

For grade 2 tumours, the sensitivity was decreased at 42.6% (−4.2%, CI:−10.6% to 2.6%), but the difference was not statistically significant. The specificity was increased at 96.2% (+3.9%,

TABLE 7 Diagnostic accuracy at the first reading (human system alone) and second reading (applying the new guidelines) for the histologic grade.

	2nd reading		2nd reading vs. 1st reading		
Grade	Sensitivity	Specificity	Difference of diagnostic accuracy: χ^2, df, *p*-value	Sensitivity	Specificity
1	95.6% (93.0%, 97.3%)	59.2% (50.7%, 67.2%)	8.5 2, 0.014	+3.9% (0.9%, 7.1%)	−3.6% (−9.1%, 2.1%)
2	42.6% (34.7%, 50.8%)	96.2% (93.5%, 97.8%)	8.8, 2, 0.012	−4.2% (−10.6%, 2.6%)	+3.9% (1.1%, 6.8%)
3	90.9% (70.9%, 97.6%)	95.1% (92.7%, 96.8%)	2.65, 2, 0.270	+10.9% (0.0%, 20.0%)	+0.6% (−1.3%, 2.3%)

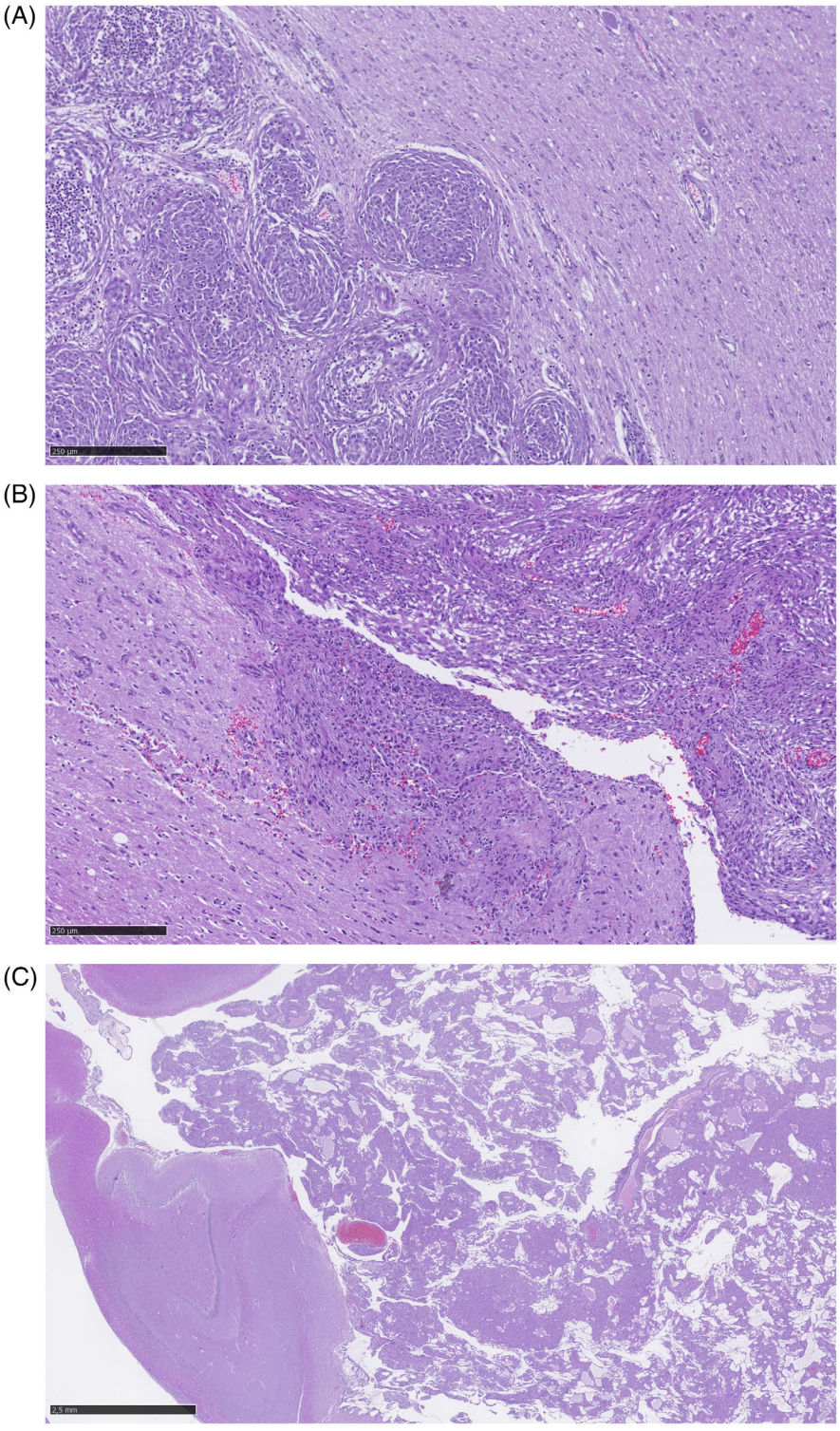

FIGURE 5 Invasion. (A) Presence of invasion. Complete agreement among surgical pathologists. Tumoral cells are not lined by normal meningeal cells and infiltrate the brain parenchyma. (B) Partial agreement among surgical pathologists. Clear meningeal delimitation of the tumour is masked by cell degeneration and multilayer fibrous reaction. (C) Absence of invasion. Complete agreement among surgical pathologists. The meningioma is well defined and almost detached by normal parenchyma. No tumour infiltration into the nervous system is observed. HE stain.

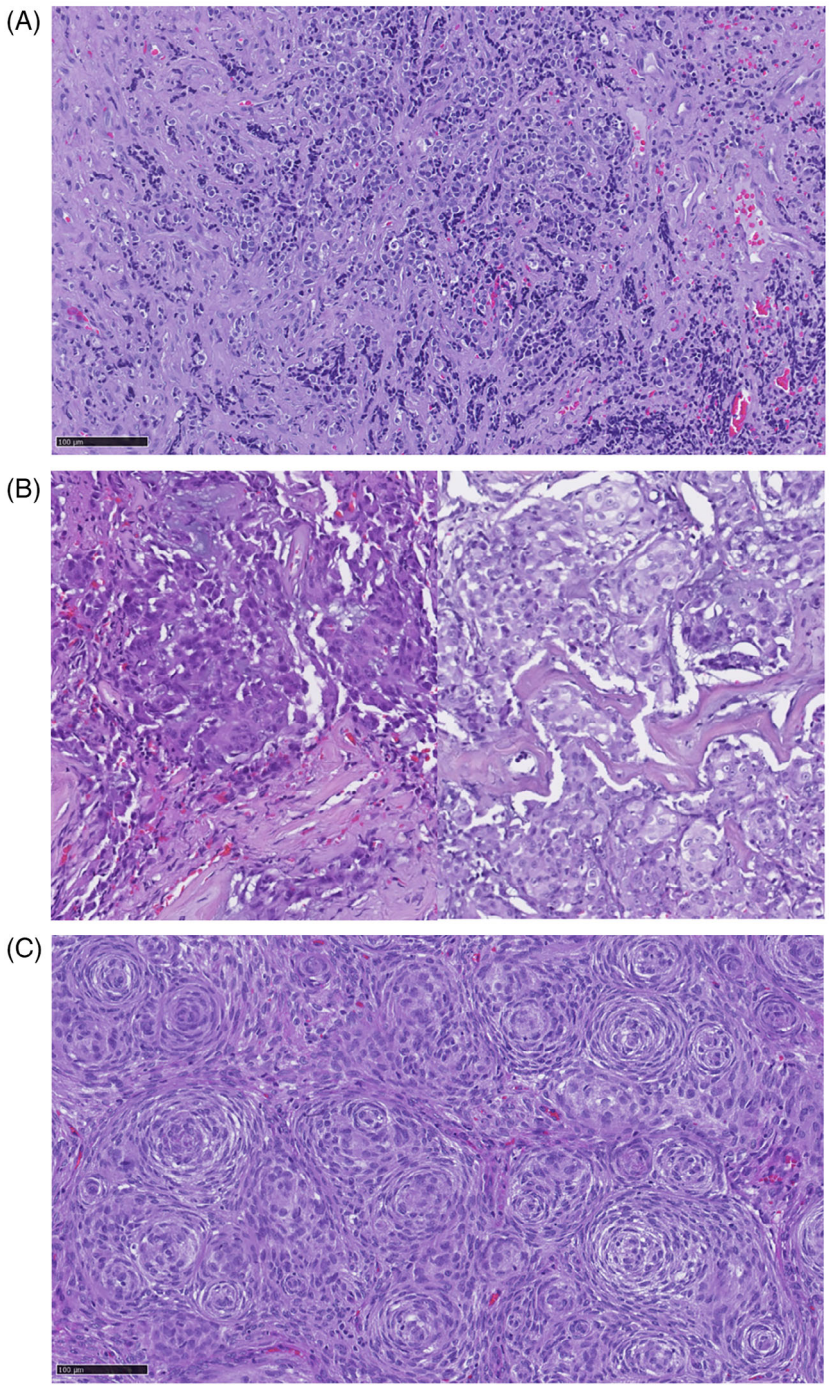

FIGURE 6 Anaplasia. (A). Presence of anaplasia. Complete agreement among surgical pathologists. In an abundant collagenous stroma, there are small cells with condensed nucleus, consistent with lymphocytes or degenerating tumoral cells, and polygonal viable tumoral cells with clear cytoplasm and finely dispersed chromatin. Tumoral cells are arranged in nests but no typical meningioma pattern is present, making meningioma diagnosis difficult. (B) Partial agreement among surgical pathologists. The tumour presents anaplastic areas, characterized by cells with severe anisocytosis and anisokaryosis (on the left), along with areas with more differentiated cells arranged in a meningothelial pattern (on the right). (C) Absence of anaplasia. Complete agreement among surgical pathologists. Tumour cells are well differentiated and arranged in whorls and bundles, typical of transitional subtype. Neither anisocytosis, nor anisokaryosis are present. HE stain.

CI: 1.1%–6.8%) and this difference was statistically significant. For grade 3 tumours, sensitivity was increased at 90.9% (+10.9%, 0% to 20%) and specificity was increased at 95.1% (+0.6%, CI: −1.3% to 2.3%), but these differences were not statistically significant.

Overall, the diagnostic accuracy of the grading systems was statistically greater when performed following the new guidelines for grade 1 and 2 tumours, and it seemed to be greater for grade 3 tumours (although not statistically significant).

4 | DISCUSSION

In canine patients, the 1-year survival rate following surgical resection of intracranial meningiomas is approximately 50%.[2,3] Canine meningiomas, as well as human meningiomas, exhibit a wide range of histologic patterns and clinical behaviour. In human patients, histopathologic findings, and, in particular tumour grade, play a key role in prognostication and therapeutic decision-making.[10] However, it is currently unknown if and how the human tumour grade could be used in dogs to predict clinical outcome

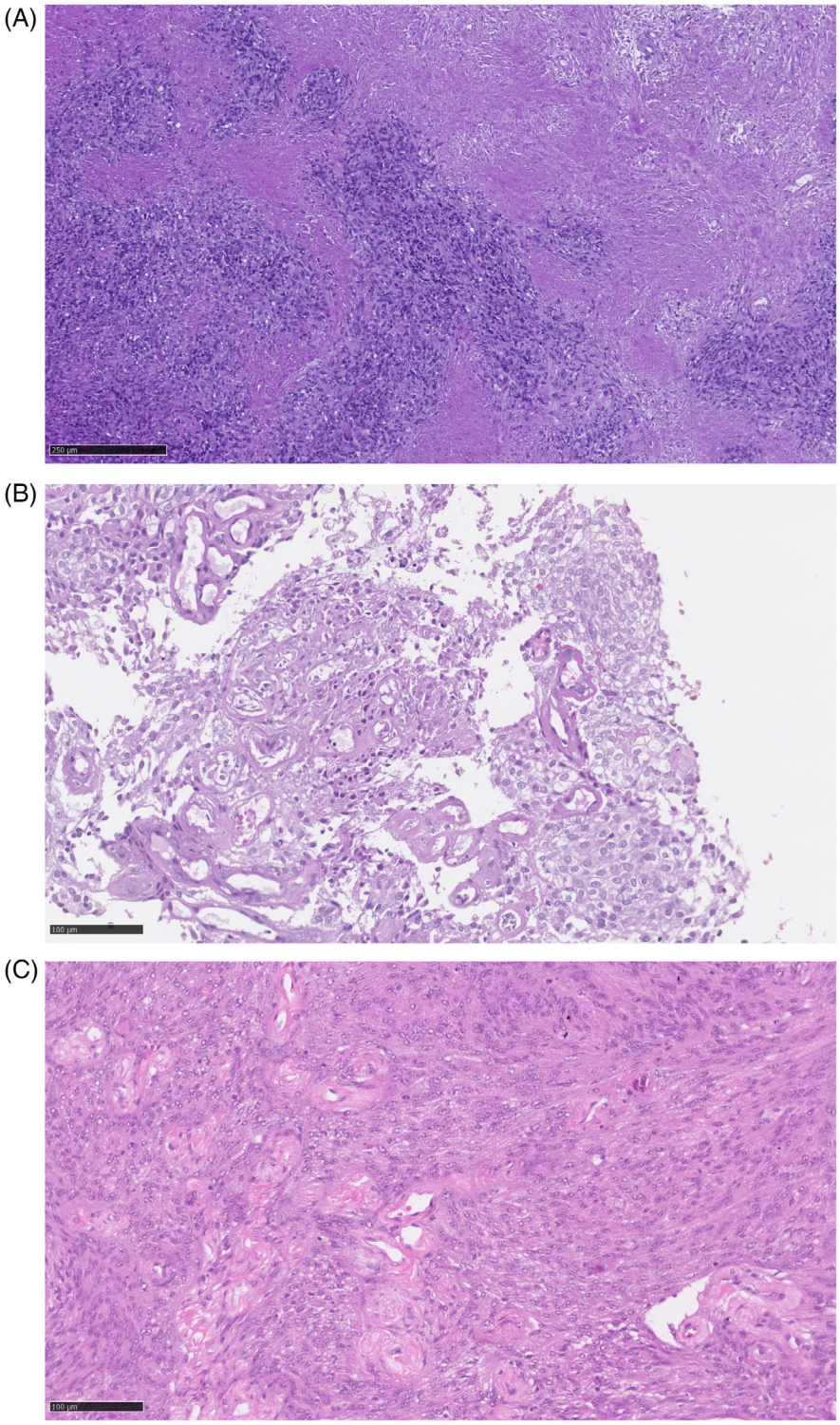

FIGURE 7 Spontaneous necrosis. (A) Presence of necrosis. Complete agreement among surgical pathologists. Large coalescent areas of necrosis, characterized by nuclear loss but preserved cellular outline (coagulative necrosis) separate small areas of viable tumour cells. (B). Partial agreement among surgical pathologists. Small areas of cellular degeneration and necrosis, characterized by loss of cellular details and presence of amorphous eosinophilic material, are scattered throughout the sample. Since the sample is composed of small fragments, interpretation of spontaneous versus artefactual necrosis was not consensual. (C) Absence of necrosis. Complete agreement among surgical pathologists. No necrotic areas are present. HE stain.

and response to treatment. Standardization of histologic grading is a prerequisite for reliable and clinically relevant prognostic studies. Previous data showed that the human histologic grading system has suboptimal reproducibility when applied to dogs,[22] far below that reported in human medicine.[12] New guidelines have been recently proposed to increase the reproducibility of the human grading system, but these guidelines have not been validated.[22] Therefore, the goal of this study was to assess the reproducibility and diagnostic accuracy of these guidelines.

4.1 | Inter-observer agreement

Histologic grade. For the histologic grade, application of the guidelines resulted in an increased inter-observer agreement shown by the kappa index, which shifted from 0.41 to 0.52. The improved agreement is comparable to that obtained in veterinary medicine with other grading systems and tumours (for gliomas $\kappa = 0.5$[19]; for canine soft tissue sarcoma $\kappa = 0.43$[20]). Nevertheless, this result is below the

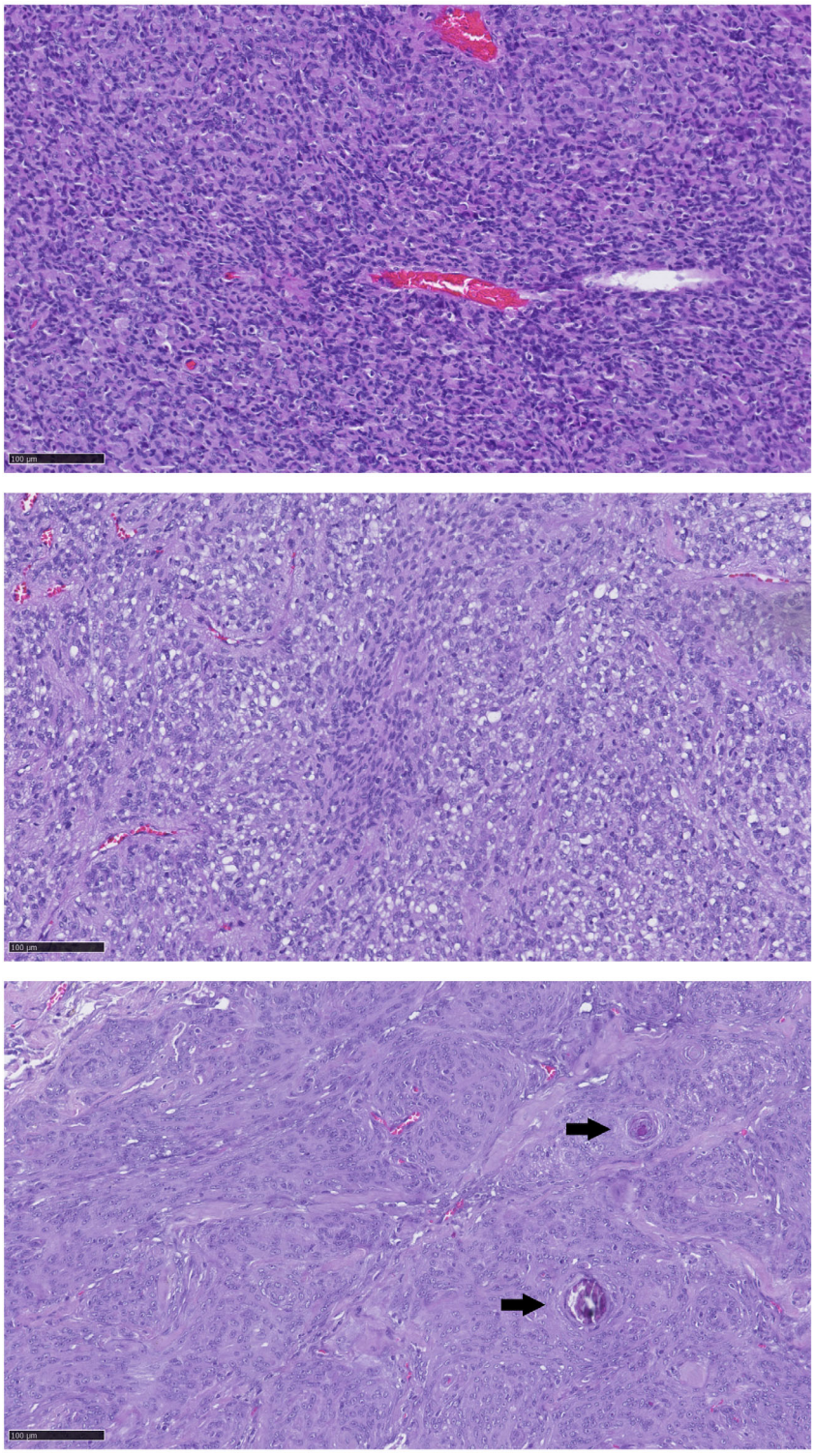

FIGURE 8 Small cells. (A) Presence of small cells. Complete agreement among surgical pathologists. The tumour is composed of small cells with increased nucleus-cytoplasmic ratio. Cytoplasm is scant and nucleus is small, often elongated and irregular. (B). Partial agreement among surgical pathologists. Throughout the tumour there are occasional clusters of small cells characterized by increased nucleus-cytoplasmic ratio. The nucleus is small and elongated. These areas were interpreted by some pathologists as artefactual or degenerative, or in a too low number to be representative of the tumour. (C) Absence of small cells. Complete agreement among surgical pathologists. The tumoral cells are moderate to large in size. Two psammoma bodies are also evident on the right (arrows). HE stain.

agreement obtained for human meningioma ($\kappa = 0.71–0.84$, depending on tumour grade).[12] The lower agreement obtained in the dog might be because the agreement for human meningioma was calculated among neuropathologists, while for our study it was calculated among surgical pathologists. In veterinary medicine, there are few specialized neuropathology laboratories, and veterinarians, especially if not board-certified in neurology, usually send all samples, including those from neurologic cases, to their preferred diagnostic laboratory. Thus, surgical pathologists working in these facilities regularly receive nervous system samples. If their diagnostic reproducibility in defining the tumour type is good,[21] they would certainly benefit from clear and precise guidelines to define tumour grade.

Histologic grade of meningiomas is defined by eight criteria, separated into main criteria and soft criteria. The three main criteria are

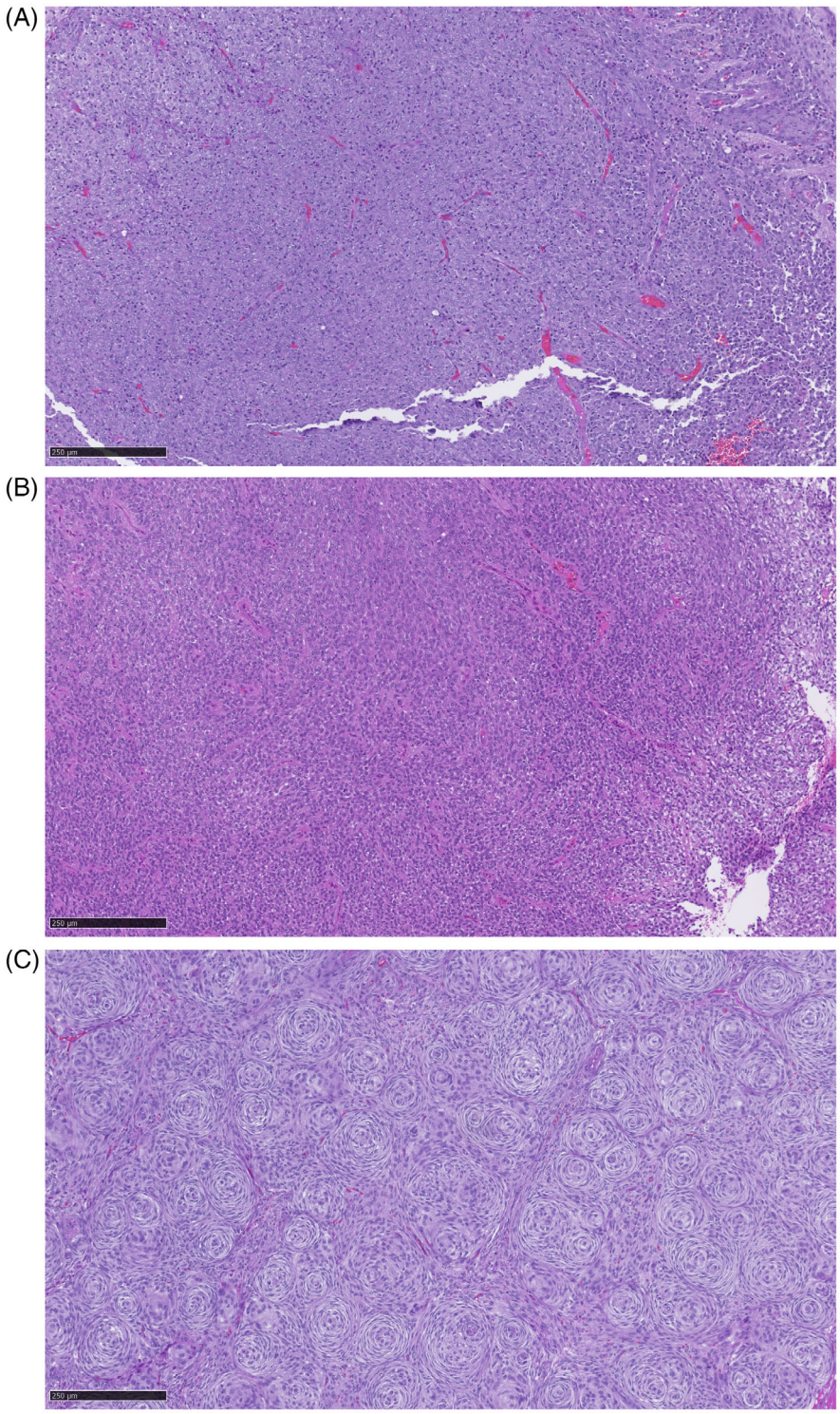

FIGURE 9 Pattern loss. (A) Presence of pattern loss. Complete agreement among surgical pathologists. Tumoral cells are arranged in sheets, with any specific cellular pattern. No streams, bundles, or whorls are present. The tumour subtype given for this tumour ranged from rhabdoid, chordoid, meningothelial and anaplastic, suggesting the lack of a clear pattern. (B). Partial agreement among surgical pathologists. Tumoral cells are arranged in sheets, but they lack atypia and other soft criteria of malignancy. At second reading, all surgical pathologists agreed about the meningothelial subtype. The lack of a specific organization in the meningothelial subtype could be interpreted as pattern loss. (C) Absence of pattern loss. Complete agreement among surgical pathologists. The neoplastic cells are clearly arranged in whorls and fascicles, typical of a transitional subtype. HE stain.

mitotic grade, invasion and anaplasia. Their presence alone is sufficient to increase the grade from 1 to 2 or 3. The five soft criteria are necrosis, macronucleoli, pattern loss/sheeting, small cells and hypercellularity. To diagnose a grade 2 tumour in the absence of one of the main criteria, the presence of at least three soft criteria is required.[5,6,10]

Main criteria. When using the guidelines, two of the three main criteria, mitotic grade and invasion, showed a substantial agreement, while anaplasia reached a moderate agreement with an increase of more than 0.2 points in the kappa index.

Mitotic count and grade. Mitotic count is often correlated with prognosis and for this reason it is integrated into most grading systems.[20,28–32] In human medicine, mitotic grade is the most important criterion for a grade 2 tumour diagnosis and it is directly related to survival.[33–35] Despite the wide use of mitotic grade, disagreements are frequently reported in veterinary medicine.[36,37] In our study,

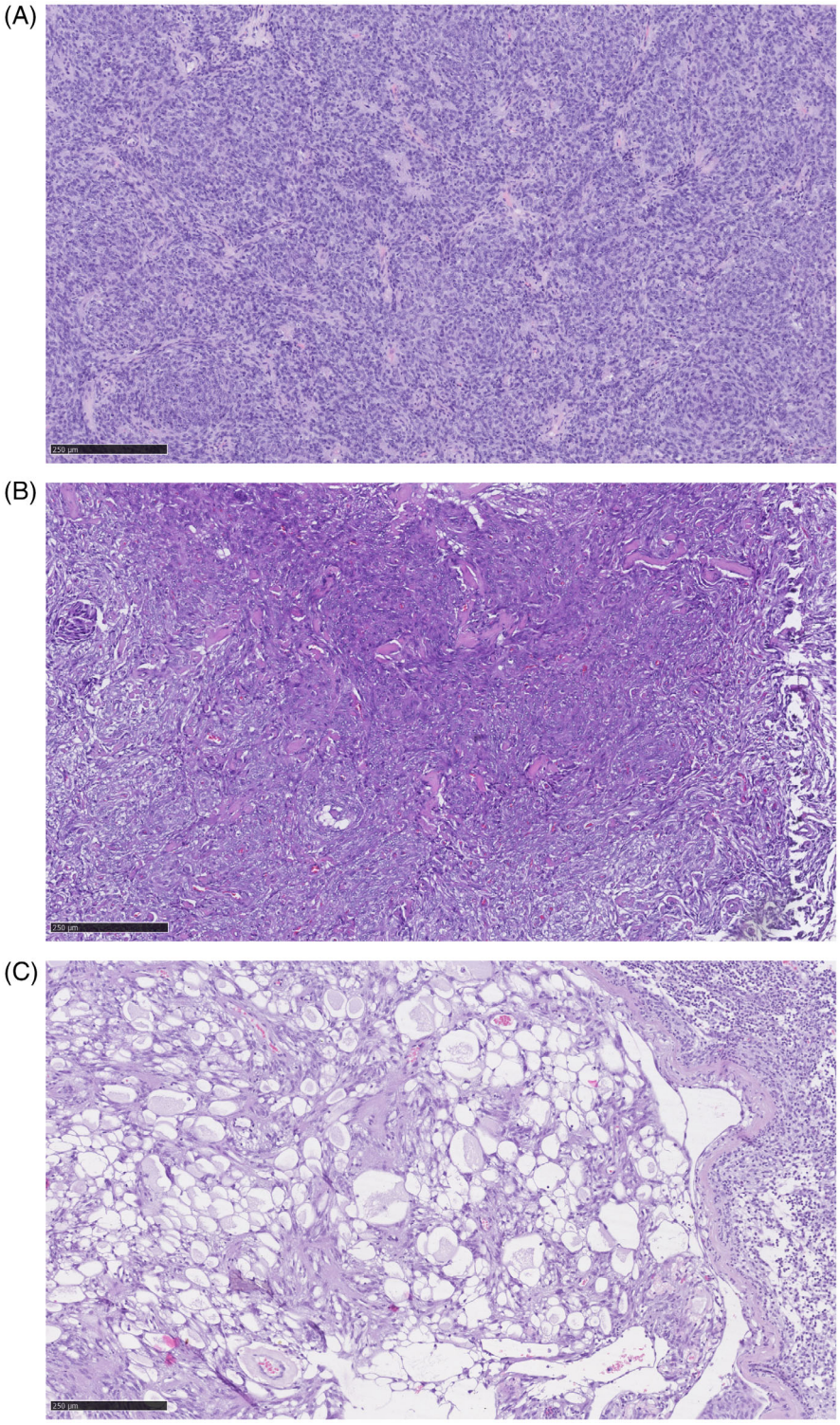

FIGURE 10 Increased cellularity. (A). Presence of increased cellularity. Complete agreement among surgical pathologists. Most of the tumour presents a high concentration of cells per unit area. Interstitial connective tissue is scant and cells are small, with almost overlapping nuclei. (B) Partial agreement among surgical pathologists. Interstitial connective tissue is scant, but cells are medium in size. (C) Absence of increased cellularity. Complete agreement among surgical pathologists. Microcystic subtype is characterized by large cells containing cytoplasmic vacuoles. The large cell size causes reduced number of cells per unit area. HE stain.

inter-observer agreement was substantial, regardless of whether the guidelines were applied, similar to that reported in human medicine ($\kappa = 0.51–0.67$).[12] Recommendations have been recently published to standardize mitotic count evaluation in veterinary medicine,[36,37] and they were integrated into the guidelines for canine meningioma grading.[22] In our study, the lack of a substantial improvement between the first and second reading may be because the surgical pathologists already applied the recommendations to standardize mitotic count during the first reading.[37]

In human grading, mitotic cut-offs were reported to be 4 and 20 mitoses in 1.60 mm.[238,39] Nevertheless, in most papers, and in the application of human grading to dog meningioma, the 1.60 mm^2 surface area was replaced by the more commonly used 10 high power fields (HPFs, old cut-off),[12,33–35,40–42] creating reduced reproducibility

in human and veterinary medicine.[22,43] In order to solve this problem, in the most recent human WHO edition, HPF is clearly replaced by surface area.[10] In the guidelines for canine meningioma grading, the human cut-offs calculated for 1.60 mm^2 were adapted to the standardized veterinary area of 2.37 mm^2 (new cut-off).[22,36,37] It is interesting to note that the new cut-off applied to canine meningiomas is more reproducible than the old cut-off. This is due to the distribution of the mitotic count in the 158 samples: 85% of the tumours had less than 8 mitoses and almost 30% of the tumours had a mitotic count around 4 in 2.37 mm^2. Thus, until otherwise proven by prognostic studies, the new mitotic cut-off should be applied to canine meningioma.

Invasion (Figure 5). In human meningioma, invasion is directly correlated with tumour relapse and poor prognosis.[11] Invasion limits the benefits of excisional surgery, since the tumour cannot be completely resected and thus will likely regrow. In the human grading system applied to canine meningiomas, invasion was not clearly defined. There was confusion about dura mater/bone invasion and brain invasion, the latter considered with or without well-defined pial delimitation of tumour cells. Therefore, the guidelines for the assessment of canine meningioma define invasion as when the pial layer does not line tumour cells apparently infiltrating the nervous tissue, as reported in the original human grading.[39] Use of this definition increased the reproducibility of invasion assessment. Nevertheless, when using the guidelines, the agreement and the kappa index are still below that reported in human medicine, where the kappa index is approximately 0.76.[12] This discrepancy could be explained by the fact that immunohistochemical markers, which are regularly used to assess invasion in human medicine, are currently lacking in veterinary medicine.

Anaplasia (Figure 6). The reproducibility of anaplasia assessment was also increased by applying the guidelines for canine meningiomas and became comparable to that obtained in human medicine.[12] Since anaplasia is a sufficient criterion to upgrade tumours to grade 3, its detection is pivotal to identify a poorer prognosis.

Soft criteria. When the guidelines were applied, agreement increased for three soft criteria (spontaneous necrosis, small cells and pattern loss). Spontaneous necrosis reached a substantial agreement. In contrast, when applying the guidelines, agreement decreased for two criteria, macronucleoli and hypercellularity.

Spontaneous necrosis (Figure 7). Evaluation of necrosis in meningioma can be challenging, since it can vary from single cell necrosis to vast areas of karyolytic and karyorrhectic debris. Moreover, iatrogenic tissue damage due to sample handling may be present, which can mimic intratumoral necrosis. Therefore, surgical pathologists with little experience in neuropathology could over-interpret this criterion. The guidelines allowed an increase in inter-observer agreement, which became a substantial agreement, comparable with that reported in human medicine for meningioma ($\kappa = 0.66$) and higher than that reported in canine soft tissue sarcomas ($\kappa = 0.46$).[12,20]

Small cells and pattern loss (Figures 8 and 9). Reproducibility was increased when small cells and pattern loss were evaluated with the guidelines, although the agreement remained fair. These results are comparable to those obtained in human medicine.[12] Pattern loss (also called "sheeting" in the human grading system) is recognized as a subjective criterion even in human medicine, causing confusion with the confluent syncytial patterns of the meningothelial subtype when observed at higher magnification.[33] Small cells are difficult to identify, since the identification of variably sized clusters of smaller tumour cells is qualitative and often subjective. Therefore, in a prognostic grading system for canine meningioma, these two criteria should be deleted, interpreted with caution or evaluated differently, to increase their reproducibility.

Macronucleoli. Reproducibility was low when assessing macronucleoli, both with and without the guidelines. In human medicine, a macronucleolus is defined as visible at 100× magnification. In our study, at 100× magnification, corrected for screen size,[22] very few to no nucleoli were visible. Moreover, the kappa index at 100× was slight, while at 200× it was fair, supporting the comment of some authors that, if really needed, nucleoli should be evaluated at 200×. Nevertheless, despite the low reproducibility, macronucleoli have a very low impact on histologic grade assessment, regardless of the magnification. Therefore, we suggest omitting macronucleoli from any future grading system correlated with prognosis for canine meningioma.

Hypercellularity (Figure 10). We found hypercellularity to be another criterion with only fair to slight reproducibility with both the human grading and when applying the guidelines. This criterion should be interpreted in relation to the mean cellularity for each specific subtype of meningioma, which remains subjective. If considered for inclusion in a new grading system for canine meningioma, then increased cellularity should be evaluated quantitatively, not qualitatively.

4.2 | Diagnostic accuracy

When applying the guidelines, grade 3 tumours were identified almost perfectly, with a sensitivity of 91% and a specificity of 95%. The increase in sensitivity and specificity when using the guidelines was not statistically significant, but this could be due to the low number of grade 3 tumours in the study. Although meningioma grades have not been fully validated and accurately linked to prognosis in dogs, several publications have associated anaplastic or grade 3 tumours with shorter survival times (less than 4 months post-surgery).[2,44,45] These data highlight the importance of an accurate diagnosis.

The guidelines also allowed better detection of grade 1 tumours, with significantly increased sensitivity, and grade 2 tumours, with significantly increased specificity. However, using the guidelines, the grade 1 specificity and grade 2 sensitivity were slightly decreased, remaining between 40% and 60%, supporting the idea that many grade 2 meningiomas can be misdiagnosed as grade 1 tumours. This is a well-known problem for three-tier grading systems,[46] supporting the proposal of two-tier grading systems for some animal tumours.[32,47]

In conclusion, the guidelines allowed an overall increased agreement among surgical pathologists as well as better detection of grade 1 and grade 3 tumours, thus they should be integrated into the current grading system for canine meningioma. Nevertheless, a low

reproducibility remains for some minor criteria and these criteria appear to have little impact in defining histologic grade. Furthermore, in human medicine, all the soft/minor criteria seem to not be significantly correlated with tumour relapse or patient survival.[34,35] Therefore, in the future, a new grading system for canine meningiomas should be developed, either avoiding or quantitatively evaluating the less reproducible histological parameters, and correlating the grade to tumour 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.

FUNDING INFORMATION

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

CONFLICT OF INTEREST STATEMENT

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

DATA AVAILABILITY STATEMENT

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

ORCID

Sara Belluco https://orcid.org/0000-0002-7209-6879
Giancarlo Avallone https://orcid.org/0000-0002-5203-7679
Fabiano José Ferreira de Sant'Ana https://orcid.org/0000-0002-3956-1329

REFERENCES

1. 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
2. Forward AK, Volk HA, Cherubini GB, et al. Clinical presentation, diagnostic findings and outcome of dogs undergoing surgical resection for intracranial meningioma: 101 dogs. *BMC Vet Res*. 2022;18(1):88. doi:10.1186/s12917-022-03182-y
3. 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
4. Koestner A. *Histological Classification of Tumors of the Nervous System of Domestic Animals*. Washington DC: Armed Forces Institute of Pathology, in cooperation with the American Registry of Pathology and The World Health Organization Collaborating Center for Worldwide reference on Comparative Pathology; 1999.
5. 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.
6. Vandevelde M, Higgins RJ, Oevermann A. Neoplasia. *Veterinary Neuropathology: Essentials of Theory and Practice*. John Wiley & Sons, Inc.; 2012.
7. 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
8. 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
9. Arie Perry DJB. Neuropathology patterns and Introduction. *Practical Surgical Neuropathology a Diagnostic Approach*. Vol 1. 2nd ed. Elsevier; 2018.
10. International Agency for Research on Cancer. *WHO Classification of Tumours. Central Nervous System Tumours*. Vol 6. 5th ed. Lyon, France: International Agency for Research and Cancer; 2021.
11. 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
12. Rogers CL, Perry A, Pugh S, et al. Pathology concordance levels for meningioma classification and grading in NRG oncology RTOG trial 0539. *Neuro-Oncol*. 2016;18(4):565-574.
13. Williams JF, Zhao M, Najdawi F, et al. Grading of medullary thyroid carcinoma: an interobserver reproducibility study. *Endocr Pathol*. 2022;33(3):371-377. doi:10.1007/s12022-022-09718-0
14. Taylor C, Puzyrenko A, Iczkowski KA. Trends in disagreement with outside genitourinary pathology diagnoses at an academic center. *Pathol Res Pract*. 2022;236:153997. doi:10.1016/j.prp.2022.153997
15. Van Bockstal MR, Berlière M, Duhoux FP, Galant C. Interobserver variability in ductal carcinoma In situ of the breast. *Am J Clin Pathol*. 2020;154(5):596-609. doi:10.1093/ajcp/aqaa077
16. Prayson RA, Agamanolis DP, Cohen ML, et al. Interobserver reproducibility among neuropathologists and surgical pathologists in fibrillary astrocytoma grading. *J Neurol Sci*. 2000;175(1):33-39. doi:10.1016/S0022-510X(00)00274-4
17. van den Bent MJ. Interobserver variation of the histopathological diagnosis in clinical trials on glioma: a clinician's perspective. *Acta Neuropathol (Berl)*. 2010;120(3):297-304.
18. Meuten DJ, Moore FM, Donovan TA, et al. International guidelines for veterinary tumor pathology: a call to action. *Vet Pathol*. 2021;58(5):766-794. doi:10.1177/03009858211013712
19. Krane GA, Shockley KR, Malarkey DE, et al. Inter-pathologist agreement on diagnosis, classification and grading of canine glioma. *Vet Comp Oncol*. 2022;20:881-889. doi:10.1111/vco.12853
20. Yap FW, Rasotto R, Priestnall SL, Parsons KJ, Stewart J. Intra- and inter-observer agreement in histological assessment of canine soft tissue sarcoma. *Vet Comp Oncol*. 2017;15(4):1553-1557. doi:10.1111/vco.12300
21. Belluco S, Avallone G, Di Palma S, Rasotto R, Oevermann A. Inter- and Intraobserver agreement of canine and feline nervous system tumors. *Vet Pathol*. 2019;56:349. doi:10.1177/0300985818824952
22. Belluco S, Marano G, Baiker K, et al. Standardization of canine meningioma grading: inter-observer agreement and recommendations for reproducible histopathologic criteria. *Vet Comp Oncol*. 2022;20:509. doi:10.1111/vco.12802
23. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics*. 1977;33(1):159-174. doi:10.2307/2529310
24. Genders TSS, Spronk S, Stijnen T, Steyerberg EW, Lesaffre E, Hunink MGM. Methods for calculating sensitivity and specificity of clustered data: a tutorial. *Radiology*. 2012;265(3):910-916. doi:10.1148/radiol.12120509
25. R Foundation for Statistical, Computing, Vienna, Austria. *R: the R Project for Statistical Computing*. Accessed June 10, 2021. https://www.r-project.org/

26. Kilem L. Gwet. *irrCAC: Computing Chance-Corrected Agreement Coefficients (CAC)*. Accessed June 10, 2021. https://CRAN.R-project.org/package=irrCAC
27. Højsgaard S, Halekoh U, Yan J. The R package geepack for generalized estimating equations. *J Stat Softw*. 2006;15:1-11. doi:10.18637/jss.v015.i02
28. Avallone G, Rasotto R, Chambers JK, et al. Review of histological grading Systems in Veterinary Medicine. *Vet Pathol*. 2021;58(5):809-828. doi:10.1177/0300985821999831
29. Dagher E, Abadie J, Loussouarn D, Campone M, Nguyen F. Feline invasive mammary carcinomas: prognostic value of histological grading. *Vet Pathol*. 2019;56(5):660-670. doi:10.1177/0300985819846870
30. Goldschmidt M, Peña L, Rasotto R, Zappulli V. Classification and grading of canine mammary tumors. *Vet Pathol*. 2011;48(1):117-131. doi:10.1177/0300985810393258
31. Spangler WL, Kass PH. The histologic and epidemiologic bases for prognostic considerations in canine melanocytic neoplasia. *Vet Pathol*. 2006;43(2):136-149. doi:10.1354/vp.43-2-136
32. Kiupel M, Webster JD, Bailey KL, et al. Proposal of a 2-tier histologic grading system for canine cutaneous mast cell tumors to more accurately predict biological behavior. *Vet Pathol*. 2011;48(1):147-155. doi:10.1177/0300985810386469
33. Backer-Grøndahl T, Moen BH, Torp SH. The histopathological spectrum of human meningiomas. *Int J Clin Exp Pathol*. 2012;5(3):231-242.
34. Karabagli P, Karabagli H, Mavi Z, Demir F, Ozkeles EY. Histopathological and clinical features as prognostic factors of atypical meningiomas. *Turk Neurosurg*. 2020;30(5):575-746. doi:10.5137/1019-5149.JTN.31161-20.1
35. Loewenstern J, Shuman W, Rutland JW, et al. Preoperative and histological predictors of recurrence and survival in atypical meningioma after initial gross Total resection. *World Neurosurg*. 2019;128:e148-e156. doi:10.1016/j.wneu.2019.04.069
36. 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
37. 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
38. Perry A. 13 – Meningiomas. In: Perry A, Brat DJ, eds. *Practical Surgical Neuropathology: A Diagnostic Approach*. 2nd ed. Elsevier; 2018:259-298. doi:10.1016/B978-0-323-44941-0.00013-8
39. 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.
40. Goldbrunner R, Minniti G, Preusser M, et al. EANO guidelines for the diagnosis and treatment of meningiomas. *Lancet Oncol*. 2016;17(9):e383-e391. doi:10.1016/S1470-2045(16)30321-7
41. Saygin I, Cakir E, Ercin ME. Interobserver variability in mitotic count for meningioma grading: how can we reduce it? *Turk Neurosurg*. 2020;30(5):643-650. doi:10.5137/1019-5149.JTN.26252-19.2
42. Salles D, Santino SF, Malinverni ACM, Stávale JN. Meningiomas: a review of general, histopathological, clinical and molecular characteristics. *Pathol Res Pract*. 2021;223:153476. doi:10.1016/j.prp.2021.153476
43. 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
44. Greco JJ, Aiken SA, Berg JM, Monette S, Bergman PJ. Evaluation of intracranial meningioma resection with a surgical aspirator in dogs: 17 cases (1996–2004). *J Am Vet Med Assoc*. 2006;229(3):394-400. doi:10.2460/javma.229.3.394
45. 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
46. Dal Col P, Garaix T, Massard A, et al. Meningioma sampling: how much is enough for the accurate grading of atypical meningiomas? *Pathology (Phila)*. 2021;20:602-607. doi:10.1016/j.pathol.2020.10.024
47. Koehler JW, Miller AD, Miller CR, et al. A revised diagnostic classification of canine glioma: towards Validation of the canine glioma patient as a naturally occurring preclinical model for human glioma. *J Neuropathol Exp Neurol*. 2018;77(11):1039-1054. doi:10.1093/jnen/nly085

How to cite this article: Belluco S, Marano G, Lurier T, et al. Standardization of canine meningioma grading: Validation of new guidelines for reproducible histopathologic criteria. *Vet Comp Oncol*. 2023;21(4):685-699. doi:10.1111/vco.12932