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DISEASE IN WILDLIFE OR EXOTIC SPECIES

Clinical, Cytological, Histological and Immunohistochemical Features of Cutaneous Mast Cell Tumours in Ferrets (*Mustela putorius furo*)

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Summary

Cutaneous mast cell tumours (cMCTs) are one of the most common cutaneous tumours in ferrets (*Mustela putorius furo*). However, limited information is available regarding cytological and histological features of these tumours and studies evaluating KIT expression are lacking in this species. The aims of this prospective study were to describe the most common clinical, cytological and histological features of cMCTs in ferrets and to compare the usefulness of different staining techniques in the diagnosis of these tumours in ferrets as well as evaluating KIT expression in neoplastic mast cells (MCs) by immunohistochemistry. Macroscopically, the tumours were small, round to plaque-like and frequently associated with surface crusting. The most common locations were the extremities and the trunk. MC granules were stained in all cases using toluidine blue (TB) and Wright–Giemsa stains in cytological specimens, but none stained with modified Wright's stain. Haematoxylin and eosin and TB on histological sections failed to stain MC granules in all the cases. Cytological and histological examination revealed low to moderate anisocytosis and anisokaryosis. An infiltrative rather than a delineated or encapsulated growth pattern was noted histologically in all cases. Eosinophilic infiltration was not uncommon and 'collagenolysis' was detected on cytological and histological examination. KIT expression was detected in all cases evaluated. In approximately one third of the cases the MCs exhibited KIT labelling pattern I and in the remaining ferrets, KIT pattern III. No correlation was found between KIT expression pattern and biological behaviour.

Keywords: cutaneous mast cell tumour; cytology; ferret; histopathology

Introduction

Cutaneous mast cell tumour (cMCT) is a common skin tumour of dogs, cats and ferrets (Rakich and Latimer, 2007; London and Thamm, 2013) and has been reported in other mammalian species, including horses, pigs, squirrels and hedgehogs (Raymond *et al.*, 1997; He *et al.*, 2009), in reptilian species such as tortoises (Santoro *et al.*, 2008), and in avian species such as agapornis (Dallwig *et al.*, 2012). There is a wide variation in location, biological behaviour and prognosis across species (Santoro *et al.*, 2008; He *et al.*, 2009). Mast cell (MC) tumours may be focal or multicentric in the skin and may also affect internal viscera such as the spleen, liver and intestine. In dogs, the behaviour and prognosis of the tumour varies according to its cytological (Camus *et al.*, 2016) or histological grade (Kiupel *et al.*, 2011), which is based on MC granularity, mitotic figures, anisokaryosis, binucleation/multinucleation and nuclear pleomorphism or the number of mitotic figures, in combination with karyomegaly,

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multinucleated cells or bizarre nuclei, respectively. There is no histological grading system or reliable prognostic marker available for cats; however, the mitotic index is correlated with the survival time (Dobromylskyj *et al.*, 2015). In other species, cMCTs range from typically benign in horses and ferrets, to commonly malignant in cattle or hedgehogs, but grading systems are also lacking for these species.

Numerous attempts have been made to apply cytological, histochemical and immunohistochemical procedures in order to achieve a definitive diagnosis and to establish a correlation with biological behaviour (Jackson *et al.*, 2013; DeNicola, 2016; Maxie and Miller, 2016). Several types of histochemical stains have been used for cytological preparations. One of the most commonly used are the Romanowsky-type stains, as they are inexpensive, readily available to the practicing veterinarian and easy to prepare, maintain and use. Romanowsky stains may be either aqueous (e.g. Diff-Quik® and Hema 3) or methanol based (e.g. Wright and Giemsa stains). However, despite the fact that aqueous-based stains are the most commonly used in private practice, they may fail to stain the granules of MCs, basophils and large granular lymphocytes (Welle *et al.*, 2008; Meinkoth *et al.*, 2014). Toluidine blue (TB) is an acidophilic metachromatic dye that selectively stains acidic tissue components. It has been used to highlight MC granules, mucins and cartilage. MC granules stain purple with TB due to the presence of heparin and histamine (Sridharan and Shankar, 2012).

Haematoxylin and eosin (HE) is the routine histological stain and the basis for comparison with other histochemical or immunohistochemical procedures. Histochemical techniques, such as use of metachromatic dyes (i.e. TB or Giemsa stain), are used to stain tissue components that cannot be distinguished or identified easily in HE-stained sections (Maxie and Miller, 2016).

Immunohistochemistry (IHC) has become a routine tool in the veterinary diagnostic laboratory with the increasing availability of antibodies that cross-react with antigens of animal species and those developed for use in veterinary medicine (Maxie and Miller, 2016). The KIT protein is a transmembrane tyrosine kinase receptor encoded by the *c-kit* proto-oncogene, which is expressed in MCs, and in other cells including basophils, melanocytes, erythroid precursors and the cells of Cajal (Kiupel *et al.*, 2004; Welle *et al.*, 2008; Ressel *et al.*, 2015). KIT has been shown to regulate normal MC survival, proliferation, differentiation and migration (Sabattini *et al.*, 2013). Mutations in *c-kit* have been identified in canine and feline cMCTs. Expression of the KIT receptor in MCTs, and its detection using IHC, has been well established in dogs, cats and horses (Kiupel *et al.*, 2004; Dobromylskyj *et al.*, 2015; Ressel *et al.*, 2015). However, interspecies differences have been found in relation to the percentage of KIT positivity, the labelling pattern and correlation between KIT expression and the biological behaviour or the histological grade.

cMCTs in ferrets (*Mustela putorius furo*) are usually considered to be clinically benign, as they do not spread locally or metastasize (Rakich and Latimer, 2007; Antinoff and Williams, 2012). They appear as small, flat or slightly raised, alopecic plaques that are variably pruritic and erythematous (Rakich and Latimer, 2007; Kanfer and Reavill, 2013). cMCTs often have overlying crusting, as a result of pruritus and self-trauma. While usually single, multiple concurrent tumours are identified occasionally. There is no age or gender predilection for development of these tumours and they may arise in any area of the body (Antinoff and Williams, 2012; Fox *et al.*, 2014). Information about the cytological and histological features of cMCTs in ferrets is scarce and studies comparing different stains, either in cytology or histology, and evaluating KIT expression in ferret cMCTs are lacking.

The aims of this prospective study were: (1) to describe the most common clinical, cytological and histological features of cMCTs in ferrets; (2) to compare the usefulness of different staining techniques in the diagnosis of cMCTs in ferrets; and (3) to evaluate KIT expression in the neoplastic MCs by IHC.

Materials and Methods

Case Selection

Ferrets with cutaneous lesions clinically compatible with cMCTs were selected from cases seen in the exotic animal service of the Fundació Hospital Clínic Veterinari of the Universitat Autònoma de Barcelona (UAB) between March 2014 and January 2015. Written informed consent was obtained from the owners before enrolment of the animal. Information regarding each ferret's clinical history including age, sex, the location and size of the nodules, the number of nodules at presentation, gross features and clinical signs were recorded.

The animals had excisional surgery (surgical margins approximately 0.5 cm) for tumour removal. Tumours were sampled by making an impression smear immediately after removal and before processing the samples for histological examination. Triplicate cytological specimens were submitted for analysis to the Veterinary Clinical Pathology laboratory at the UAB. Histological samples were fixed in 10% neutral buffered formalin and submitted to the Veterinary Pathology laboratory at the UAB.

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Follow-up information was gathered from recheck consultations and/or telephone interviews with owners. Information regarding disease progression, recurrence or appearance of new masses was recorded. The patient status during follow-up was recorded as alive, dead because of non-tumour-related causes or dead because of tumour-related causes.

Cytological Evaluation

Impression smears were processed routinely and stained with modified Wright's (MW), TB and Wright–Giemsa (WG) stains. Cases were included if an adequate cellular sample was obtained for each stain.

MW staining was performed manually according to the manufacturer's recommendations. Each slide was immersed five times in each solution, with approximately 1 s between each dip. TB staining was performed according to the protocol described by Lastra *et al*. (2015). TB solution was prepared by dissolving 0.5 g of TB in 20 ml of 95% ethanol and adding 80 ml of distilled water. The staining solution was filtered and stored in a dark bottle at 4°C. The staining protocol was: TB solution 1–2 min, followed by three dips in distilled water. WG staining was performed according to the protocol described by Campbell (2015b). WG stain was prepared by dissolving 300 mg of powdered Wright's stain and 30 g powdered Giemsa stain in 100 ml of absolute methanol. The staining solution was stored in a tightly sealed brown bottle for 2 days and it was filtered. The buffer solution was prepared by dissolving 3.80 g Na_2HPO_4 (dibasic sodium phosphate) and 5.47 g KH_2PO_4 (monobasic potassium phosphate) in 500 ml of distilled water, and then distilled water was added to make a total volume of 1.0 l. The staining protocol was: imprint smears were flooded with WG stain for 1–3 min and then an equal amount of buffer was added and mixed by gently blowing on the slide until a metallic green sheen formed on the surface. This stood for 2–6 min and then the smears were gently rinsed with distilled water.

A range of morphological parameters was assessed and classified, including MC granularity for the three stains (i.e. mild, moderate or marked), nuclear pleomorphism (i.e. absent or present), anisokaryosis/anisocytosis (i.e. absent or present), binucleation or multinucleation (i.e. absent or present), mitotic figures (i.e. absent or present), 'collagenolysis' (i.e. absent or present) and eosinophilic infiltration (i.e. absent, mild, moderate or marked), and the final diagnosis obtained with each stain.

Nuclear pleomorphism was recorded as present if non-rounded nuclear shapes were present and absent if only round to oval shapes were noted. Anisokaryosis was defined as >50% variation in nuclear size.

Histological Evaluation

Histological samples were processed routinely, fixed in 10% neutral buffered formalin and embedded in paraffin wax. Sections (3 μm) were stained with HE and TB. For TB staining, blue toluidine at 1% was applied for 20 min and the samples were then washed with distilled water.

A range of morphological parameters were assessed and classified, including completeness of excision (i.e. incomplete or complete), cellularity (i.e. mild, moderate or marked), growth pattern (i.e. sheets, cords or nests), MC granularity with both stains (i.e. mild, moderate or marked), anisokaryosis/anisocytosis (i.e. mild, moderate or marked), nuclear pleomorphism (i.e. absent or present), mitotic index, 'collagenolysis' (i.e. absent or present) and eosinophilic infiltration (i.e. absent, mild, moderate or marked) and the final diagnosis obtained with each stain.

Margins were incomplete if the tumour extended to a margin and complete if all margins were ≥2 mm. Nuclear pleomorphism was recorded as present if non-rounded nuclear shapes were seen and absent if only round to oval shapes were noted. Anisokaryosis was defined as >50% variation in nuclear size. The mitotic index was established as the average of the number of mitotic figures per 10 randomly chosen representative high-power fields (HPFs; ×400).

Immunohistochemistry

KIT expression was evaluated by IHC with anti-KIT antibodies using the PT-Link automatic System (Dako, Glostrup, Denmark) for dewaxing, rehydration and epitope retrieval. IHC was performed on a Dako Autostainer Plus (Dako) using procedures, buffers and solutions provided by the manufacturer. The rabbit/mouse EnVision™ detection system (Dako) was used at the dilution recommended by the manufacturer. After washing, slides were incubated for 5 min in chromogen (3,3′ diaminobenzidine with H_2O_2) (Dako) to reveal binding. After washing, slides were counterstained in Mayer's haematoxylin for 10 s, washed in running tap water and then dehydrated, cleared and mounted. KIT antigen was demonstrated using a rabbit polyclonal antibody against human CD117 (Dako) diluted at 1 in 400. Sections of normal skin containing some non-neoplastic MCs and sections from normal jejunum (containing cells of Cajal) obtained from a ferret served as positive controls for KIT expression.

Three different patterns of KIT expression were determined according to the literature for canine

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cMCTs (Kiupel *et al.*, 2004): (1) pattern I, MCs express KIT mainly on the cell membrane with only minimal cytoplasmic labelling; (2) pattern II, MCs express KIT in the cytoplasm, primarily adjacent to the nucleus; the labelling pattern is either intense, focally clustered or strongly positive with stippling; and (3) pattern III, MCs express KIT in the cytoplasm, the labelling pattern is diffuse, obscuring all of the other cytoplasmic features.

Data Evaluation and Statistical Analysis

All cytological specimens were reviewed in blinded fashion by two board-certified pathologists (AM and JP). All histological sections were reviewed by a 3rd-year anatomical pathology resident (GD) and a board-certified anatomical pathologist (AR) without knowledge of the prior cytological or histological findings. After comparison of the results, a common final assessment was undertaken, which resulted in an agreement on the rare discordant cases. The data were summarized by use of descriptive statistics (median and percentages). Analyses were performed using SPSS version 19.0 statistical software (SPSS Inc., Chicago, Illinois, USA).

Results

Clinical Findings

Sixteen nodules were excised surgically from 11 ferrets. One was excluded from the study as it was a sebaceous epithelioma. Fifteen cMCTs from 10 ferrets were included in the study, and histological (15/15) and cytological (12/15) examinations were performed.

The age of the affected ferrets ranged from 2 to 9 years (median 5 years). Seven ferrets were neutered males and three were neutered females. At the time of presentation, seven ferrets had a solitary cMCT (70%), two ferrets had two cMCTs (20%) and one ferret had four cMCTs (10%). The cMCTs were located in the extremities (8/15, 53.3%), the trunk (5/15, 33.3%) and the head or neck (2/15, 13.3%).

Most of the ferrets had no clinical signs associated with the tumour (80%), but two animals showed pruritus. Macroscopically, the tumours were small (1–4 mm in diameter) and round to plaque-like. The majority of the tumours (9/15 cases, 60%) were associated with surface crusting. Cutaneous erythema was seen in six animals (40%) and alopecia in two animals (13.3%) (Fig. 1).

Cytological Findings

In 12 tumours, adequate samples were obtained for cytological evaluation with all three stains. MC granules were not visualized with MW stain in any case. Evaluation of the same samples with TB and WG stains revealed marked MCs granulation in 10 cases (83.3%) and moderate granulation in three cases (25%), with a 100% agreement between both stains. In cases in which MC granulation was marked, the nuclei of the cells were obscured by the granules in the cytoplasm. On cytological examination, cMCTs stained with MW showed variable cellularity. Cells were arranged singly and in non-cohesive aggregates with well-defined cell borders. The cytoplasm was clear and finely vacuolated or granular. The nuclei were round and placed paracentrally, the chromatin pattern was finely stippled to coarse and nucleoli were seen rarely. Moderate anisocytosis and anisokaryosis were seen in one case and nuclear pleomorphism was identified in two cases (Fig. 2). Binucleated cells were seen in one case and mitotic figures were not found in any case. Mild eosinophilic infiltration was seen in one case and marked eosinophilic infiltration in another. 'Collagenolysis' was seen in three cases (25%) (Fig. 3). A definitive diagnosis of cMCT was achieved in all cases with TB and WG stains, while a diagnosis of round cell neoplasia was made with MW stain.

Histological and Immunohistochemical Features

Histological examination of sections stained with HE showed no MC granulation in any case. The cytoplasmic granularity of the MCs was not clear with TB stain. In some cases, the results were considered equivocal by the two pathologists, as no clear purple or blue granules could be detected in the cytoplasm. An increase in staining time did not improve the TB results and, therefore, TB-stained sections were considered negative in all cases.

In all skin samples a neoplastic round cell proliferation was observed, expanding the superficial dermis and, in some cases, reaching the deep dermis. These masses were non-delineated, non-encapsulated and infiltrative, and extended between the collagen bundles of the dermis. Tumour cellularity ranged from mild (in the case of superficial growth, 6.7% cases) to moderate–marked (in the case of infiltrative growth to the deep dermis, 93.3% cases). The growth pattern was in sheets (nine cases), cords (two cases), nests and cords (two cases) and in sheets and cords (two cases). Proliferating cells were round, with well-defined cytoplasmic margins and a moderate amount of basophilic cytoplasm. Nuclei were central, with finely stippled chromatin. Nuclear pleomorphism was absent in all cases and anisocytosis/anisokaryosis was mild in eight cases (53.33%) and moderate in seven cases (46.7%). The mitotic index

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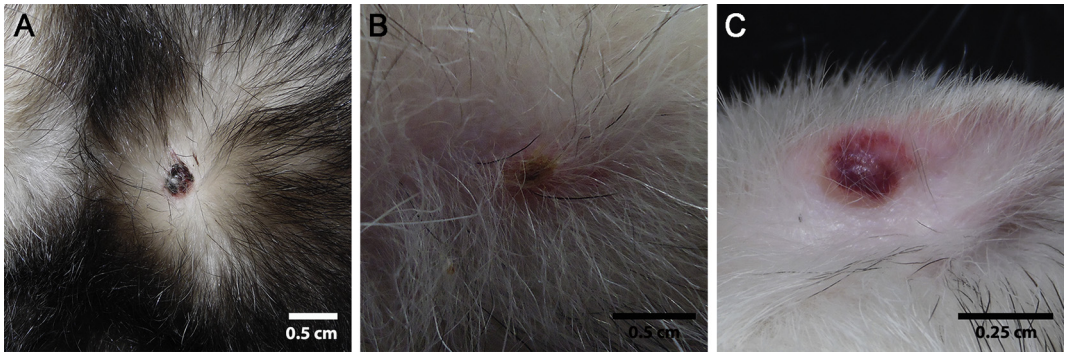

Fig. 1. Macroscopic appearance of cMCTs in ferrets. The nodular masses ranged between 1 and 4 mm in diameter. (A) Tumour associated with black surface crusting; usually this presentation indicated self-trauma due to pruritus. (B) Tumour associated with yellow crusting. (C) Erythematous tumour.

was low in all cases (≤1 mitosis/HPF). In seven cases (46.7%) scattered eosinophils were recognized among the neoplastic MCs and five cases (33.3%) showed a moderate eosinophilic infiltration. 'Collagenolysis' was detected in four cases (26.7%) (Fig. 4). Excisional surgery was complete in 11 ferrets (73.3%) and incomplete in four cases (26.7%). The difficulty in observing granules in the cytoplasm of neoplastic cells in the HE- and TB-stained samples hindered the final diagnosis, and only a diagnosis of round cell neoplasia was achieved with both stains.

KIT was expressed by normal MCs in the positive control sections (as well as the cells of Cajal in the jejunum) and the MCs in all cMCTs. The neoplastic MCs in five cases (33.3%) had mainly cell membrane labelling with minimal cytoplasmic expression (pattern I). The same pattern was found in the normal MCs from the positive controls. In 10 cases

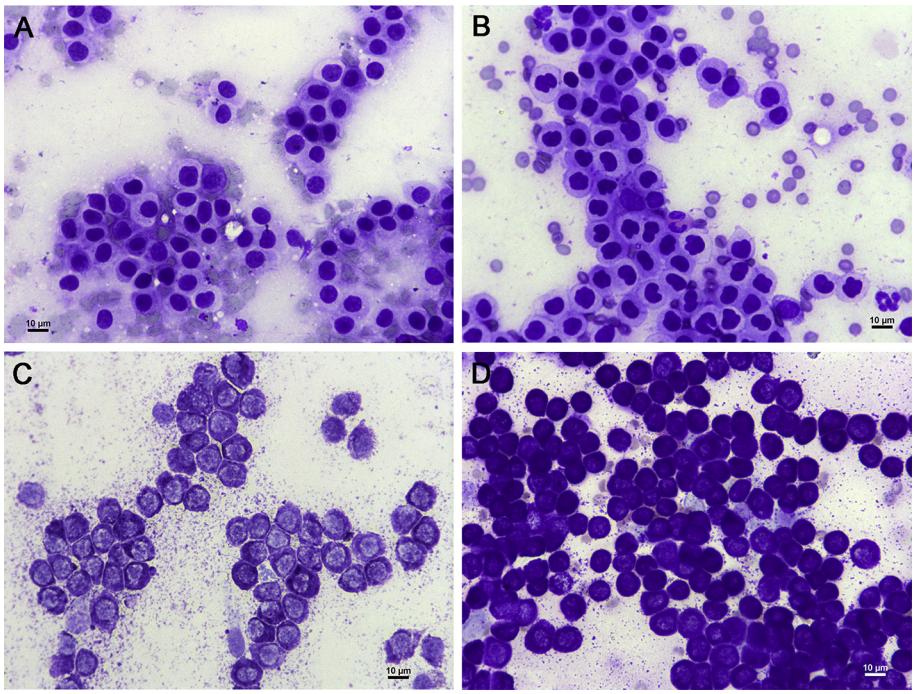

Fig. 2. Impression smears from ferret cMCTs. (A) Modified Wright's stain. Cells are arranged singly and in non-cohesive aggregates with well-defined cell borders. They have light blue and finely vacuolated cytoplasm. Anisokaryosis/anisocytosis are low. Intracytoplasmic granules are not stained. (B) Moderate nuclear pleomorphism and binucleated cells seen in one case. Modified Wright's stain. (C) Toluidine blue stain. (D) Wright–Giemsa stain. With both stains in (C) and (D), purple to blue intracytoplasmic granules are observed.

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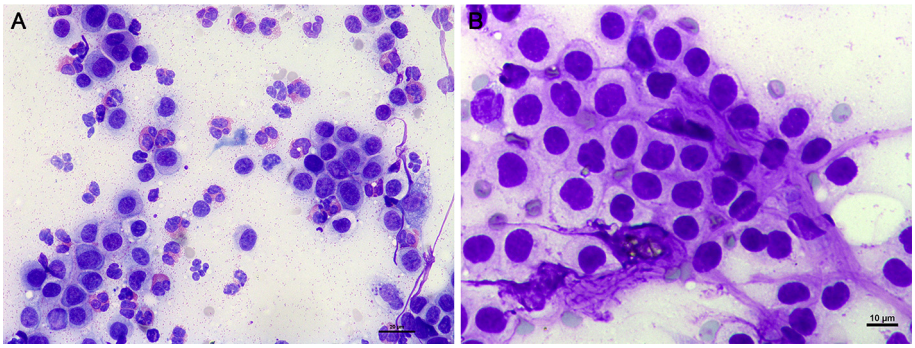

Fig. 3. Impression smears from ferret cMCTs showing (A) eosinophilic infiltration and (B) 'collagenolysis'. Modified Wright's stain.

(66.7%), neoplastic MCs showed diffuse cytoplasmic labelling, obscuring all other cytoplasmic features (pattern III) (Fig. 5). KIT IHC allowed the final diagnosis of cMCT in all cases.

Complete follow-up information was obtained in nine cases (90%). The median follow-up time was 12 months (1–27 months). No recurrence or metastatic disease was reported in any case. One animal developed a new cutaneous nodule (at another site relative to the previous one), which showed spontaneous regression (histological examination was not performed). Three animals were humanely destroyed due to developing an insulinoma (1, 5 and 24 months after surgery). Post-mortem examination was performed in all three cases and no tumour recurrence or metastases were detected. One patient was humanely destroyed due to severe liver disease 24 months after the initiation of the study. At the time of writing, four animals are alive with no recurrence or new tumours.

Discussion

The present study has investigated the clinical, cytological and histological features of cMCTs in ferrets and their staining characteristics and KIT expression pattern.

The average age of the affected ferrets was 5 years, similar to that recorded in the literature. The wide age range confirmed that cMCTs can develop in ferrets at any age. No gender predilection has been reported in the literature (Fox *et al.*, 2014), and even though we found male ferrets to be more frequently affected than females, this could be due to the low number of animals included in the study. Thirty percent of the ferrets had more than one cMCT. Multiple MCTs have been reported previously in ferrets (Fox *et al.*, 2014), as in dogs (between 5 and 25% of cases) (Welle *et al.*, 2008) and cats (Sabattini *et al.*, 2013). The most common locations of cMCTs in the present study were the extremities and the trunk. Macroscopic characteristics of cMCTs and clinical signs were in accordance with most published data.

Cytologically, MC granules were stained with TB and WG in all cases; however, MW failed to stain the granules in all cases. Anecdotal descriptions of failure to stain the granules in some cMCTs using aqueous-based Romanowsky stains such as Diff-Quik® in cytological preparations have been reported in ferrets (Campbell and Grant, 2010; Campbell, 2015a), but not with a methanol-based Romanowsky stain such as MW. Failure to stain granules of MCs using Diff-Quik® is reported widely in dogs, making accurate identification of MCTs from other discrete round cell tumours difficult or impossible (DeNicola, 2016). A recent study refutes the claim that prolonged fixation time improves staining of MC granules with Diff-Quik® stain in canine patients (Jackson *et al.*, 2013).

Histologically, MCs granules were not stained in any case with HE or TB. In cats, the same feature has been observed; although granules are abundant structurally, an obvious granularity is infrequent even with metachromatic stains (Mauldin and Peters-Kennedy, 2016). In dogs, HE stain is able to detected intracytoplasmic granules in histological preparations, except in poorly differentiated cMCTs containing few intracytoplasmic granules. In these cases, TB is the stain of choice to demonstrate the granules. In the present study, the use of TB staining was inconclusive in all cases because, even when using immersion oil, the presence of cytoplasmic granules was doubtful in all of the tumours. Varied MC granularity and no dominant pattern with TB stain have been described in horses (Ressel *et al.*, 2015). The reason for the discrepancy observed in our study between the effectiveness of TB in cytology and histology is unknown.

Knowing the different staining characteristics of MCTs is important to avoid misdiagnosis or inaccurate tumour classification. Non-staining granules can lead to incorrect diagnosis of other round cell

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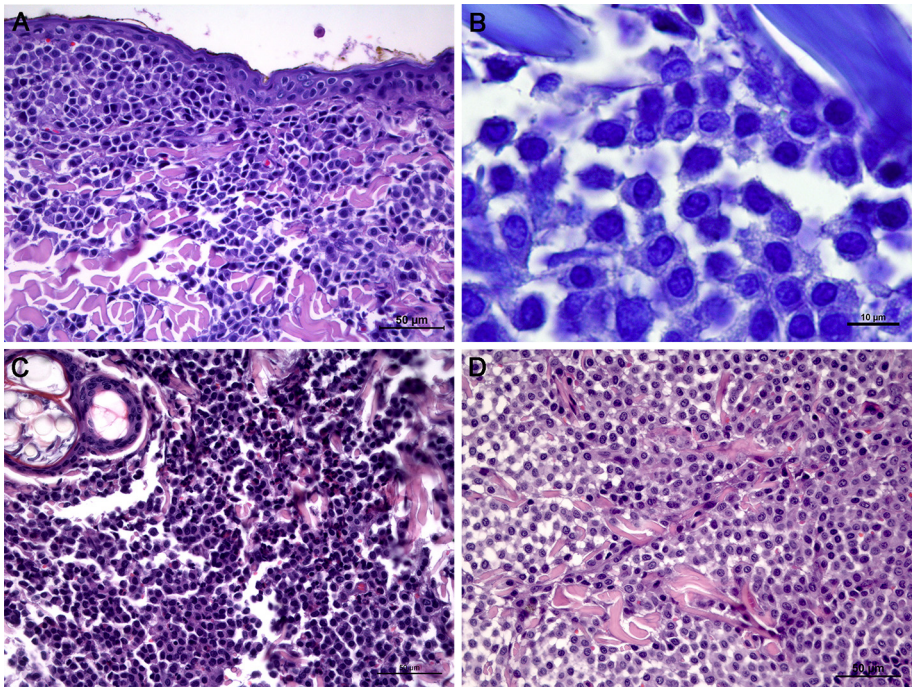

Fig. 4. Histological sections from ferret cMCTs. (A) Neoplastic round cell proliferation with infiltrative behaviour, expanding the upper layers of the dermis. Granules of the neoplastic mast cells are not recognized. HE. (B) The neoplastic mast cells do not exhibit clear cytoplasmic granules with TB stain. (C) Presence of eosinophils between the neoplastic cells. HE. (D) Multifocal dermal collagen fibres show hyaline change ('collagenolysis'). HE.

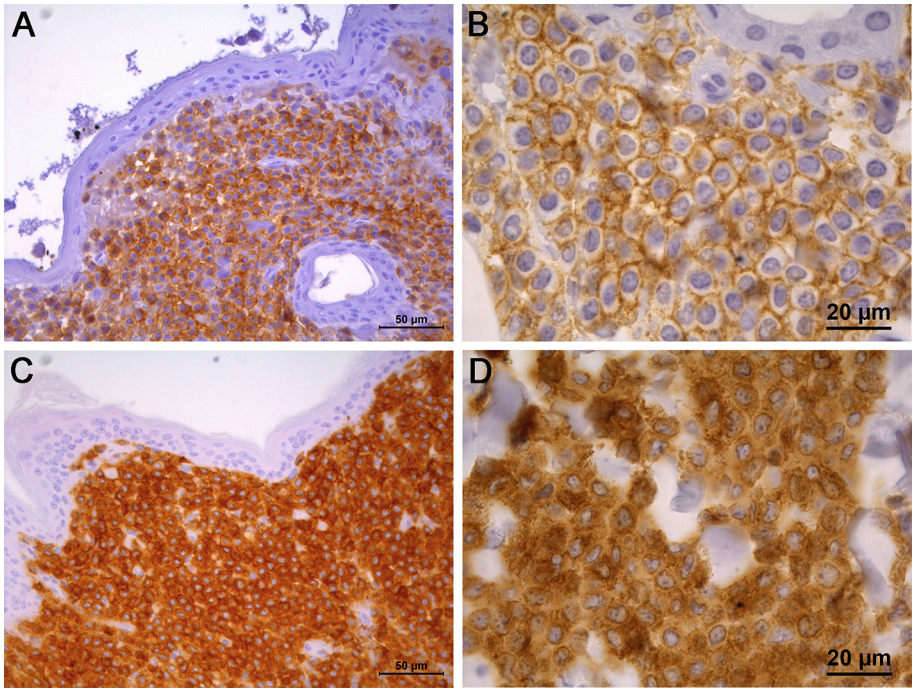

Fig. 5. (A) Mast cells exhibit membrane expression of KIT. IHC. (B) Higher-power view of the membrane labelling (pattern I). IHC. (C) Mast cells showing cytoplasmic KIT expression. IHC. (D) Higher-power view of diffuse cytoplasmic labelling (pattern III). The immunoreaction obscures other cytoplasmic features. IHC.

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tumours such as histiocytoma or epitheliotropic lymphoma, instead of MCT. Weakly-stained granules can lead to classification of the tumour as an undifferentiated MCT, implying a worse prognosis, when this is simply a staining effect.

General cellular morphological characteristics of cMCTs in ferrets are similar to those described in other species. However, some authors describe some particular characteristics of ferret cMCTs, such as mild or moderate amounts of granules (Rakich and Latimer, 2007; Kanfer and Reavill, 2013; Fox *et al.*, 2014), an uncommon eosinophilic infiltration (Parker and Picut, 1993; Rakich and Latimer, 2007), absence of 'collagenolysis' (Parker and Picut, 1993; Fox *et al.*, 2014) or a well-circumscribed to mild infiltration of the superficial dermis (Parker and Picut, 1993; Antinoff and Williams, 2012). In contrast, our cytological results revealed that the majority of cMCTs had a marked amount of intracytoplasmic granulation. Some authors have described MC degranulation as a possible cause of this typical mild granularity (Rakich and Latimer, 2007); however, if that were the case, the granules would have not stained with TB or WG stains. It is possible that the staining method used in the previous reports might have contributed to the variation in the granulation observed, since, as has been demonstrated in the present study, some stains fail to detect MC granules in ferrets.

Eosinophilic infiltration was detected in approximately 75% of the cases in the histological examination, being mild in 50% of these cases. Although dogs typically have numerous eosinophils admixed with the MCs and the number of eosinophils was lower in ferret cMCTs compared with those of dogs, the presence of eosinophilic infiltration should not be considered uncommon. 'Collagenolysis' was detected in approximately 25% of cases in cytological and histological examination and to our knowledge, this is the first report of possible degenerate collagen fibrils in ferret cMCTs. Histological examination revealed a non-delineated, non-encapsulated neoplastic proliferation with infiltrative behaviour in >90% of cases, extending to the deep dermis, a feature which is not in accordance with the previously published literature.

KIT expression was demonstrated by IHC in all the cases in the present study. To our knowledge, this is the first description of KIT expression in cMCTs in this species. KIT expression has been demonstrated in normal cells from ferrets such as interstitial cells of Cajal, MCs and spermatocytes as well as neoplastic cells in a gastrointestinal stromal tumour and in a mixed germ cell–sex cord stromal tumour (Girard-Luc *et al.*, 2009; Inoue *et al.*, 2015). In our study, one third of the cases had pattern I labelling, while two thirds of the cases expressed pattern III. The normal MCs of the skin (positive controls) exhibited pattern I labelling.

The KIT receptor is a transmembrane protein and, as such, immunoreactivity of this protein is located in the cell membrane of non-neoplastic mast cells. In dogs, aberrant cytoplasmic KIT expression has been associated with tumour recurrence and shorter survival time (Welle *et al.*, 2008; Sabattini *et al.*, 2013). A correlation between the expression and labelling pattern of the KIT receptor and the histological grade of MCTs has been made in dogs, with well-differentiated tumours weakly expressing KIT with a membrane labelling and poorly differentiated tumours having a high expression of KIT with a cytoplasmic labelling (Kiupel *et al.*, 2004). In cats the relationship between KIT expression and clinical outcome is more complex and not completely understood; a recent study found a significantly lower survival time in cats expressing cytoplasmic KIT labelling. However, KIT positivity varies in feline cMCTs from 69 to 92.6% of cases (Dobromylskyj *et al.*, 2015). In equine cMCTs, a correlation between KIT expression and morphological parameters of malignancy was found, but this was not correlated with clinical outcome or aggressive behaviour due to the lack of available data (Ressel *et al.*, 2015).

No ferret in the present study experienced tumour recurrence, aggressive behaviour or died from causes related to the cMCT, not even those cases with incomplete surgical margins or KIT pattern III. While completeness of surgical excision is predictive of outcome in canine cMCTs, the same does not appear to be true in cats, and this could also occur with ferrets. In cats, numerous reports have failed to find a correlation between completeness of excision and recurrence rate (Henry and Herrera, 2013). Additionally, it has been proven that feline KIT protein can be present in the cytoplasm in some cases, without any correlation with biological behaviour, and the same appears to be the case in ferrets. One possible explanation for this finding in cats is that the KIT receptor is actively synthesized in the cytoplasm before it migrates to the cell membrane (Rodriguez-Cariño *et al.*, 2009).

In conclusion, the diagnosis of cMCTs in ferrets must be supported by complementary TB or WG stains in cytological preparations and/or KIT IHC in histological specimens. Eosinophilic infiltration and 'collagenolysis' may be associated with cMCTs in ferrets. Histologically, cMCTs are non-encapsulated with an infiltrative behaviour. KIT

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cytoplasmic expression pattern is not related to the biological behaviour of the tumour in ferrets, as has been observed in other species.

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