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Lucie Chevallier, Amaury Briand, Edouard Reyes-gomez, Wilfried Defer, Yves Millemann, Jérémy Béguin

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CASE REPORT

Clinical, pathologic, and molecular characterization of a non-metastatic multicentric cutaneous mast cell tumor in a cow

Lucie Chevallier[1] | Amaury Briand[2] | Edouard Reyes-Gomez[1,3] | Wilfried Defer[4] | Yves Millemann[4,5] | Jérémy Béguin[6,7]

[1]INSERM, UPEC, Ecole nationale vétérinaire d'Alfort, U955—IMRB, Team 10 - Biology of the Neuromuscular System, Maisons-Alfort, France

[2]Ecole Nationale Vétérinaire d'Alfort, CHUV-Animaux de Compagnie, Unité de Dermatologie, Maisons-Alfort, France

[3]Ecole Nationale Vétérinaire d'Alfort, Biopôle Alfort, Laboratoire d'anatomo-Cytopathologie, Maisons-Alfort, France

[4]Ecole Nationale Vétérinaire d'Alfort, CHUV-Animaux de Production, Maisons-Alfort, France

[5]ANSES, Laboratoire de Sécurité des Aliments, Maisons-Alfort, France

[6]Department of Internal Medicine, Ecole Nationale Vétérinaire d'Alfort, CHUV-Animaux de Compagnie, Maisons-Alfort, France

[7]INRAE, ANSES, Ecole nationale vétérinaire d'Alfort, UMR Virologie, Maisons-Alfort, France

Correspondence
Lucie Chevallier, Ecole nationale vétérinaire d'Alfort, Maisons-Alfort, France.
Email: lucie.chevallier@vet-alfort.fr

Abstract

Cutaneous mast cell tumors are rarely reported in cattle. Although mutations in the *c-KIT* gene have been shown to play a central role in the oncogenesis of canine mast cell tumors, few data are available in cattle. This report describes the clinical, histologic, immunohistochemical, and genetic features of a multicentric cutaneous mast cell tumor in an adult cow. An 11-year-old Prim'Holstein cow was presented for a 5-month history of multiple skin nodules. Cytologic and histologic analyses of the nodules led to a diagnosis of mast cell tumors. Immunohistochemical analysis for KIT expression showed a moderate to strong signal in neoplastic mast cells with a cytoplasmic and membranous pattern. Sequencing of the *c-KIT* gene coding sequence revealed no mutation. Despite partial response after corticosteroid treatment, euthanasia was elected. No metastases to the lymph nodes, spleen, and liver were identified at post-mortem and histologic examinations.

KEYWORDS
cattle, *c-KIT* oncogene, genetics, histology, skin tumor

Cutaneous mast cell tumors (MCTs) are the most common cutaneous neoplasm in dogs, accounting for 16%–21% of skin neoplasms in this species.[1] Some breeds, such as Boxers, Boston terriers, English bulldogs, Pugs, Labradors, and Chinese Shar-Peis, are at increased risk. MCTs are usually solitary cutaneous masses; however, 11%–14% are presented with multiple lesions.[2] Mutations in the *c-KIT* gene, mostly internal duplications in exons 11 and 12, have been shown to play a central role in canine MCT oncogenesis.[3–6] These tumors are histologically graded using 3-tier and 2-tier grading systems.[7,8] Prognostic factors include clinical features such as clinical stage or anatomic site, histological grade, proliferation markers such as Ki67 index, immunohistochemical pattern of KIT expression, and the presence of activating mutations in *c-KIT*.

In cattle, MCTs are less frequently reported and represent less than 1% of all neoplasms.[9] MCTs are either reported as multiple cutaneous lesions distributed over the entire body with or without internal organ involvement or as single visceral lesions.[9–11] Because of the limited follow-up and available data regarding therapeutic options in this species, the progression of the disease is not well characterized. Pathologic findings, in particular the *c-KIT* mutation, and

expression, are less documented than in dogs. Only one case report described a diffuse cytoplasmic expression of KIT in a multicentric cutaneous MCT in a cow, but *c-KIT* mutations were not assessed.[11]

This report describes the clinical, histologic, immunohistochemical, and genetic findings of a multicentric cutaneous MCT in an adult cow treated with corticosteroids.

An 11-year-old Prim'Holstein cow was presented with a 5-month history of cutaneous nodules without other abnormalities. No other cow in the herd presented these lesions. The cow was in good body condition, and all vital signs were within normal limits at the clinical examination. Numerous cutaneous nodules were noticed over the whole body; some were ulcerated (Figure 1A). No superficial lymphadenopathy was identified on clinical examination.

Fine-needle aspirations of two nodules were performed, and smears were stained with May-Grünwald & Giemsa. Cytologic examination revealed a high number of mast cells with moderate anisocytosis and anisokaryosis and an abundant cytoplasm containing numerous metachromatic granules (Figure 2). Occasional binucleated mast cells were present. Other cells include numerous eosinophils and a few macrophages and fibroblasts. Cytology was consistent with a diagnosis of multicentric MCT. A histopathologic examination was performed on three nodules fixed in 10% buffered-formalin and stained with hematoxylin–eosin and saffron (HES). Histologically, the masses were composed of a dermal proliferation of mast cells. The nuclei were ovoid with clumped chromatin and 1 or 2 small nucleoli. Necrosis and mitoses were rare (<1 mitosis per 10 high-power fields, corresponding to 3.07 mm^2) (Figure 3). Cutaneous adnexa were disrupted by the neoplastic proliferation. An eosinophilic infiltrate was present, as well as scattered areas of eosinophilic degranulation. The final histologic diagnosis was multicentric cutaneous MCTs. Expression of KIT was assessed immunohistochemically on one representative cutaneous nodule and a fragment of normal skin (defined as a sample without gross and microscopic lesions) from the same animal.

Briefly, 5-µm-thick sections were placed on Superfrost Plus microscopy slides (Thermo Fisher Scientific). Automated immunohistochemistry was performed on the Discovery ULTRA system (Roche, Ventana Medical Systems Inc.). Slides were deparaffinized in an aqueous-based detergent solution (Discovery Wash, Ventana). Heat-induced antigen retrieval was performed using CC2 solution (Discovery Cell Conditioning Solution 2 pH 6.0) at 95°C for 8 min then at 100°C for 12 min. Primary antibody CD117 (RB9038, polyclonal, 1:200, Thermo Scientific) was incubated at 37°C for 32 min. The secondary antibody (OmniMap anti-Rabbit HRP, Roche) was incubated at 37°C for 16 min. The immunoreaction was revealed with a ChromoMap DAB Kit (Roche) and counterstained with hematoxylin. A section from a canine MCT, known to express CD117, as well as non-neoplastic normal mast cells in the tested sample and normal skin from this animal, were used as positive control and positive internal control, respectively. A negative control was assessed using phosphate-buffered saline instead of the primary antibody. KIT expression was detected in both neoplastic and normal skin mast cells at moderate to high levels (Figure 4). Normal mast cells, recognized on normal skin and differentiated from neoplastic mast cells by their morphology (smaller with a lesser amount of cytoplasm and smaller nucleus with denser chromatin) and their distribution

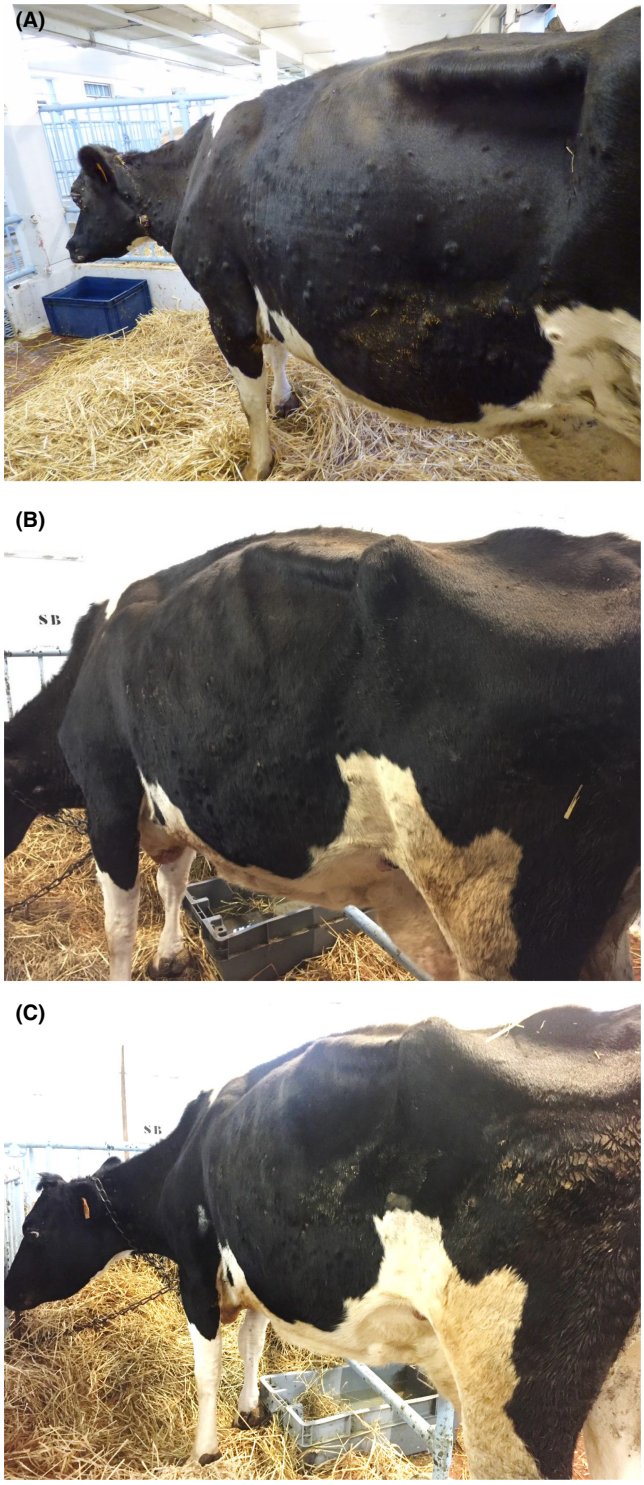

FIGURE 1 (A) Multicentric cutaneous mast cell tumors in a Holstein cow before treatment. (B) Partial response after corticosteroid treatment on day 7 (a mean reduction in the size of 10 target lesions of 35% compared with that at diagnosis). (C) Partial response after corticosteroid treatment on day 14 (a mean reduction in the size of 10 target lesions of 35% compared with that at diagnosis).

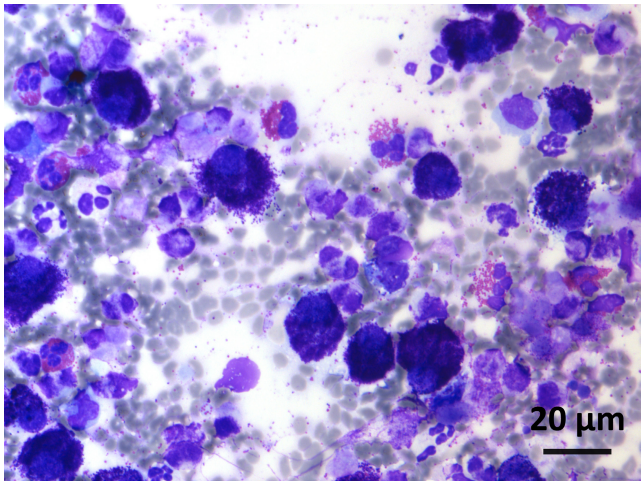

FIGURE 2 Representative field of a cytologic smear obtained after fine-needle aspiration of a nodule showing mast cells with metachromatic granules, eosinophils, and few neutrophils and macrophages. Scale bar = 20 µm, May-Grünwald Giemsa stain.

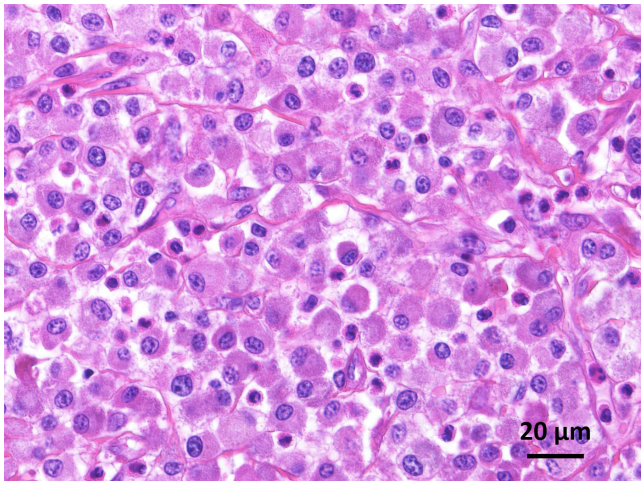

FIGURE 3 Representative histopathological section of a cutaneous nodule showing moderately pleomorphic mast cells with some eosinophils. Scale bar = 20 µm, HES stain.

(scattered through the dermis, usually around vessels, without forming clusters) mainly expressed a membranous pattern with minimal cytoplasmic staining.[12] Neoplastic mast cells had both marked granular cytoplasmic and membranous staining.

Treatment was initiated with daily intravenous injection of dexamethasone (Dexadreson, Intervet) at 0.1 mg/kg/day for 1 week and tapered to 0.05 mg/kg/day for 6 weeks. Response to treatment was assessed according to RECIST criteria. Target lesions were defined as measurable lesions at the time of diagnosis (minimum ≥ 10 mm). Ten target lesions were measured (length and width) weekly for 7 weeks to characterize the tumor response. Complete response was defined as the disappearance of all target lesions. Partial response was characterized by at least a 30% reduction in the sum of the diameters of the target lesions. Stable disease was defined as no more than a 20% increase or 30% decrease in the sum of the diameters of the

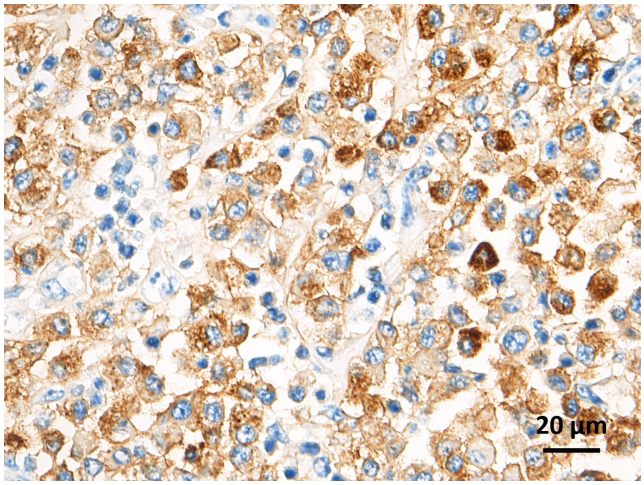

FIGURE 4 Representative immunohistochemical staining for KIT showing granular cytoplasmic and membranous expression. Scale bar = 20 µm, KIT immunohistochemical stain.

target lesions. Progressive disease was defined by the appearance of one or more new lesions or at least a 20% increase in the sum of the diameters of the target lesions.[13] Improvement was noticed within the first weeks of treatment (Figure 1B,C). At weeks five and seven, complete remission was noticed for two lesions out of 10 and a partial response for eight out of 10.

Euthanasia was elected by the owner for financial restraints. At necropsy, apart from the cutaneous nodules, other significant gross lesions included multiple abomasal ulcers and renal calculi with slight hydronephrosis. No visceral metastases were identified on gross examination. Liver, kidney, spleen, gallbladder, lung, bone marrow, left mandibular and right precrural lymph nodes, and multiple cutaneous nodules were sampled for histologic analysis. As *c-KIT* mutations are commonly reported in humans and dogs affected with MCT, sequencing of the coding sequence of *c-KIT* proto-oncogene was performed. The presence of mutations was evaluated by sequencing cDNA obtained from MCT nodules and normal skin. RNA was extracted from the tumor, and normal skin tissue was fixed in RNA*later* solution (Thermo Fisher Scientific) using a phenol-chloroform protocol.[14] RNA quantity and quality were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific). RNA quantity was over 1000 ng/µL for each sample, and A260/280, and A260/230 ratios were >2. RNA integrity was tested on 2% agarose gel, and two clear and strong bands corresponding to 28S rRNA and 18S rRNA were visualized at 4–5 and 2 kb, respectively, depicting good-quality RNA. For each sample, 1 µg of mRNA was reverse transcribed using Maxima First Strand cDNA Synthesis Kit for RT-qPCR (ThermoFisher Scientific). *C-KIT* cDNA was amplified by PCR using 4 primer pairs (Eurofins Genomics) that were designed to amplify overlapping sequences of the *c-KIT* gene and cover the complete coding sequence of the gene. The two first primer pairs covered the gene sequence coding for the extracellular domain (1F- CTCTTCGTTCTGCTGCTCCT and 1R- GTCACCCTGATGCCAGCTAT; 2F- AAAGGAAAACAGCCAGCAGA

and 2R- GAACTCTTGCCCACATCGTT) while the two last primer pairs covered the end of the extracellular domain, the juxtamembranous domain and the tyrosine kinase domain (3F- AGCA CCATTGATGACAGCAC and 3R- TGTCTGCCTTGGTTGGTACA; 4F- TTCAAAGGAGTCTTCCTGCAA and 4R- GACATCTTCGTG GACAAGCA). PCR was carried out in an Eppendorf Mastercycler Nexus Thermal Cycler thermocycler (Eppendorf AG), with maintenance at 95°C for 5 min, then 30 cycles of 94°C for 30 s for denaturation, 60°C for 40 s for annealing, 72°C for 40 s for extension, and finally maintained at 72°C for 5 min for molecular stabilization. Amplified products of respectively (1) 840, (2) 900, (3) 900, and (4) 834 base pairs were visualized by electrophoresis on a 2% agarose gel, purified with the NucleoSpin Gel and PCR Clean-up Kit (Macherey-Nagel) and were then sequenced by Sanger sequencing (Eurofins Genomics). Obtained sequences from *c-KIT* cDNA from normal and tumoral skin were compared with the reference *c-KIT* coding sequence (transcript ID: ENSBTAT00000003498.6) downloaded from the Ensembl genome browser (ensembl.org). Sequences were aligned using the Multalin alignment tool.[15]

C-KIT cDNA transcripts from normal skin and MCT displayed no mutation compared to the reference cDNA sequence, except for a 12 base pairs (bp) deletion at the end of exon 9 that was present in both normal and tumoral skin. To evaluate the presence of this 12 bp deletion in germline DNA, PCR amplification was performed using germline DNA extracted from several tissues (spleen, liver, skin, and blood) and using a primer pair designed to flank the deletion (DEL-1F- GGGCCAGTGGATGTACAGAT in exon 9 and DEL-1R- gccagtgatggaatggactt in intron 10). Sequencing revealed that the 12 bp deletion detected in the *c-KIT* skin transcript was not present in germline DNA. Sequencing of the end of exon 9 in germline DNA extracted from the blood of three other healthy Holstein cows did not show any deletion either.

1 | DISCUSSION

In this report, we describe a non-metastatic case of multicentric cutaneous MCT with histologic, immunohistochemistry for CD117 (KIT), and genetic characterization. To our knowledge, this is the first case report of an MCT in a cow that includes screening for mutations in the *c-KIT* of neoplastic mast cells.

Mast cell neoplasia is characterized by abnormal mast cell proliferation and is well-documented in humans, dogs, and cats. Nevertheless, MCTs are rarely described in cattle. In dogs, breeds such as Boxers and other breeds of bulldogs appear to be more predisposed to MCT. As in our case, most published cases are described in Holstein cattle; however, no breed predisposition has been reported. Most cases of bovine MCTs are multicentric. The skin appears to be the most common site with single or multiple nodules. Unlike our case, other organs involved include lymph nodes, liver, spleen, lung, skeletal muscle, heart, kidney, omentum, pleura, pericardium, peritoneum, tongue, and uterus.[16,17,9,18,19,11]

KIT is a tyrosine kinase membrane receptor encoded by the *c-KIT* gene that plays important roles in the maintenance and proliferation of different types of cells, including mast cells. In dogs, expression of the KIT receptor in MCTs and detection of the KIT receptor using immunohistochemistry is routinely performed. However, immunohistochemical characterization with CD117 (KIT) expression in cutaneous MCT of cattle has only been described once with diffuse cytoplasmic expression. In our case, membranous and cytoplasmic expression were identified. In dogs, various patterns of KIT expression have been reported. Normal mast cells and some neoplastic mast cells express KIT mainly on the cell membrane, whereas in many neoplastic mast cells, KIT accumulates in the cytoplasm, primarily adjacent to the nucleus. Localization of KIT expression has been correlated with biological behavior, and cytoplasmic staining patterns are associated with higher grades.[20,21] In horses, aberrant cytoplasmic KIT staining did not seem to be correlated with malignant disease or a worse prognosis.[22] As no histological grading has been reported for MCT in cattle, such a correlation could not be performed in our case. However, low mitotic count, rare multinucleated cells, and the absence of metastasis on post-mortem examination led us to suspect a less aggressive behavior.

Mutations in the c-*KIT* gene are common molecular abnormalities involved in mast cell tumorigenesis and occur in 8%–29% of canine cutaneous MCT, 56–68% of feline cutaneous and splenic MCT, 44% of human pediatric mastocytoses, and less than 5% of adult human systemic mastocytosis.[23] The primary mutations associated with MCT development in those species involve exons 8, 9, and 11.[23] In dogs, tandem duplications, point mutations, and small deletions have been shown to result in KIT auto-dimerization and autophosphorylation, even in the absence of its ligand, promoting tumorigenesis.[5,24,25]

To our knowledge, our study is the first to investigate a *c-KIT* gene mutation in a cow with MCT. We investigated the whole coding sequence of the *c-KIT* gene, as no specific exons of the gene are known to be mutated in cattle. We did not detect any mutation in the coding sequence of the *c-KIT* gene except for a 12 bp deletion at the end of exon 9 in the transcript. Nevertheless, this absence was not found on germline DNA, and a truncation of the exact same four amino acids had already been described as normal alternative splicing in the dog, the pig, and the horse on the Ensembl genome browser (ensembl.org). We hypothesize that this variation leads to a non-pathologic protein isoform in cattle and is not related to MCT development.

Nevertheless, it cannot be ruled out that a mutation in a regulatory sequence of the *c-KIT* gene exacerbates this phenomenon. *c-KIT* gene mutations outside the coding sequence have not been investigated in our study. Such a mutation could explain an overexpression of the *c-KIT* gene in the mast cells that would promote their abnormal proliferation.

In addition, intronic variations in the *c-KIT* gene in dogs have been attributed to increased tumor development and reduced survival.[26] Thus, complete sequencing of the *c-KIT* gene in the tumor would be of interest. Detailed evaluation of *c-KIT* gene sequence integrity may be clinically helpful to justify the use of tyrosine kinase inhibitors or, more widely to help select anti-neoplastic drugs.[27] Another phenomenon that could increase KIT expression without direct mutation of the gene is an autocrine or paracrine production of KIT ligand (stem cell factor) that may contribute to the growth and survival of MCT.[28]

At this level, the genetic investigation could not explain the observed translocation of the KIT receptor from the plasma membrane to the cytoplasm in neoplastic tissue, as shown by immunostaining. Other genetic mechanisms have been reported to be implicated in canine MCT, such as mutations in the epigenetic regulator *TET2* gene, the cellular signaling *GNAI2* gene, and various hyaluronidase genes involved in cancer metastasis. Finally, epigenetic changes, particularly hypomethylation, have been reported in high-grade and poorly differentiated MCTs.[23] Further studies are needed to characterize genetic alterations in cattle diagnosed with MCT.

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CONFLICT OF INTEREST STATEMENT

Authors declare no conflict of interest.

ORCID

Lucie Chevallier https://orcid.org/0000-0001-8602-1993

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