CUTANEOUS AND SYSTEMIC PYOGRANULOMATOUS REACTION FOLLOWING ADMINISTRATION OF A SPOT-ON FORMULATION OF AN INSECTICIDE - A CASE REPORT

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ABSTRACT

An eight year old female spayed 5 kg mix breed dog was presented with a 3 month history of a skin ulcer that first occurred 5 days after administration of an imidacloprid spot-on formulation. The dog also had erosive and crusted lesions on the dorsum and head, a firm nodule on the tongue and an ocular infection. The rest of the physical examination was un-remarkable. Blood work showed a neutrophilia, hypoalbuminemia, hypercholesterolemia and an elevated alkaline phosphatase activity. Serum cortisol levels were 4.74 μg/dl before and 20.6 μg/dl after ACTH administration. Cytology of the skin lesions showed neutrophils, macrophages and cocci. Biopsy showed a pyogranulomatous dermatitis and panniculitis. The dog's general condition deteriorated and it died. Necropsy revealed pus in the subcutaneous adipose tissue and the mesentery, and a pyogranulomatous reaction in the lungs, liver and heart. No organisms were detected with special stains. To the best of the author's knowledge this is the first reported observation of a possible association between imidacloprid application and a pyogranulomatous reaction.

Key words: dog, granuloma, spot-on

INTRODUCTION

Cutaneous reactions to drugs are common in dogs and very frequent in humans. These are immunologic and non-immunologic reactions which may be related to the pharmacologic actions of the drug and to the administered dose - predicted reactions. There are also unpredictable drug reactions, which are idiosyncratic. The immunologic reactions include types I-IV hypersensitivity and other unknown immunologic mechanisms. Although certain drugs are more frequently associated with drug eruptions, any drug can cause an eruption (1).

Sterile granuloma and pyogranuloma is an idiopathic uncommon condition in dogs and is diagnosed after the eliminating an infectious agent etiology. Sterile pyogranuloma is considered to be an immune-mediated condition (2). To the best of the author**'s** knowledge granulomatous reaction has not been reported as adverse drug effect.

Imidacloprid is a neonicotinoid anti-flea product. It blocks the post-synaptic acetylcholine receptors thus causing the death of the flea. It is available as a spot-on application product (Advantage® Bayer) and it has been found to be a very efficacious as well as a safe anti-flea product to be used on dogs and cats (3, 4).

The case presented here describes a pyogranulomatous reaction that developed in a dog following administration of an imidacloprid anti-flea spot-on product. To the best of the author's knowledge this is the first reported observation of a possible association between imidacloprid application and a pyogranulomatous reaction.

CASE REPORT

An eight year old spayed female 5 kg mixed-breed dog was presented to the dermatology department at the Veterinary Teaching Hospital of the Koret School of Veterinary Medicine of the Hebrew University of Jerusalem, Israel (D-K-VMTH). The chief complaint was a non-healing wound on the dorsal neck. Three months prior to presentation to the D-K-VMTH the dog had an imidacloprid anti-flea spot-on product (Advantage® 100 Bayer Animal Health , Germany) applied to its dorsal neck. It was the first time the owners had used this particular anti-flea product. Five days after administration a skin laceration occurred at the exact location where the product had been applied. The wound was debrided, sutured, and treated with antibiotics (cephalexin, Ceforal, Teva, IL) three times by the local veterinarian. The skin sutures however dehisced after each procedure and no improvement of the lesion was noticed after 3 months of treatment with topical iodine solution and chloramphenicol ointment (Synthomycine Abic, Israel). The dog was also treated systemically with Ofloxacin (Oflodex, Dexon, Israel) 20 mg/kg PO q 24 h and the ocular infection was treated locally with chloramphenicol eye ointment (Synthomycine, Rekah, Israel) for the 3 weeks prior to presentation at the D-K-VMTH. On presentation to the D-K-VMTH the dog had a normal appetite, but was polydypsic and lethargic. Physical examination and vital signs were normal except for an ocular discharge and the inflammatory skin lesions. A skin ulcer with granulation tissue 10cm x 5cm on the dorsal neck with irregular borders was present (Figure 1). After irrigating the skin with saline a small number of crusted lesions on the dorsum and one on the head of up to 2 cm in diameter that became erosive were observed (Figure 2). An area of edematous fragile skin that tore easily was noticed above the right hip (Figure 3). A firm nodule was noticed on the tongue (Figure 4, a & b). Cytological examination of the skin lesions on the neck showed a pyogranulomatous reaction containing neutrophils, macrophages and cocci, with

neutrophils and cocci on the dorsum and head lesions. Fine needle aspiration from the nodule on the tongue revealed neutrophils and macrophages. A complete blood count revealed leukocytosis, neutrophilia, monocytosis, and microcytic, normochromic anemia (Table 1), while serum biochemical analysis revealed hypoalbuminemia, hypercholesterolemia, hypertriglyceridemia, and an increased activity of alkaline phosphatase (ALP) (Table 2). Urinalysis values were all within normal limits. Abdominal ultrasonography showed an enlarged liver with multifocal hypoechoic areas that were interpreted as multifocal hepatic nodules. The left adrenal gland was normal in size and shape and the right adrenal gland was undetectable. Kidneys and urinary bladder appeared normal.

Irrigation of the skin**,** skin biopsy samples for histopathological evaluation**,** culture and susceptibility (C&S)**,** and a fine needle aspirat**e** of a liver nodule were performed under general anesthesia. General anesthesia was induced and maintained under the following protocol: pre-medication: acepromazine maleate (PromAce inj., Fort Dodge, Iowa, USA) at 0.02mg/kg IM , pethidine (Dolestine, Teva, Israel) at 5mg/kg IM, induction with propofol (Baxter, Ca, USA) at 2mg/kg IV and diazepam (Assival, Teva, Israel) at 4 mg/kg IV and maintenance with isoflurane (Oroka, Israel) via an endotracheal tube.

Fine needle aspirates from a hepatic nodule showed a few aggregations of histiocytes with normal nucleus to cytoplasm ratio and 1-3 nuclei per cell, as well as neutrophils, pro-and meta-myelocytes. This was interpreted as extra-medullary hematopoeisis. Histopathology of the ulcerated and erosive skin lesions showed a pyogranulomatous dermatitis and panniculitis (Figure 5). No organisms were observed on H&E and PAS stains. C&S results of the skin lesions showed *Staphylococcus pseudointermedius* and *Klebsiella spp* sensitive only to lincomycin and amikacin respectively.

The polydipsia and increased ALP activity and the hypercholesterolemia raised the suspicion of hyperadrenocorticism. An adrenocorticotropic hormone (ACTH) stimulation test was performed that was suggestive but not diagnostic of hyperadrenocorticism (baseline cortisol values– 4.74 µg/dl, post-ACTH – 20.6 µg/dl. Baseline reference range values: 2-4 µg/dl).

No improvement was noticed after 5 days of antibiotic treatment based on susceptibility results (Dalacin C, Pfizer, France, at 10 mg/kg q 24 h). The dog's general condition deteriorated, it became inappetent and died one week after antibiotic treatment was initiated.

Necropsy showed pus widespread throughout the subcutaneous adipose tissue and mesentery, necrotic tissue in the tongue nodule (Figure 4b) and near the right adrenal, multifocal nodules in the lungs, liver and heart (Figures 6-8). Histopathology of these nodules was consistent with pyogranulomatous reaction with the presence of neutrophils and histiocytes (Figures 9-10). No organisms were detected with Giemsa, PAS, silver, Gram or acid-fast stains.

DISCUSSION

Pyogranulomas in dogs are uncommon and may be caused by mycobacteria (*Mycobacterium tuberculosis, M. lepraemurium* and opportunistic or atypical mycobacteria), actinomycosis, actinobacillosis, nocardiosis, and subcutaneous or systemic mycoses (2). In the current case histopathology did not show any microorganisms using special stains. Neither serology nor polymerase chain reaction (PCR) were performed for any of the above mentioned organisms, thus their presence cannot be completely ruled out. Nevertheless the clinical and histological findings (e.g., neutrophilia, pus and necrotic tissue noticed on necropsy) are somewhat consistent with inflammation, possibly due to an infectious agent. In Israel, mycobacterial and deep mycotic infections are rare in small animals. Leishmaniosis, although endemic in parts of Israel (5), is not common in the dog's environment, nonetheless, this option cannot be excluded as a recent study pointed out an association between leishmaniosis and granulomas in dogs from endemic areas (6).

Sterile granuloma and pyogranuloma is an idiopathic uncommon condition in dogs and is diagnosed after eliminating an infectious agent etiology. Sterile pyogranuloma is considered to be an immune-mediated disease (2). An immune-mediated etiology is possible in this case if an immune-mediated adverse drug reaction to the insecticide or an irritant contact dermatitis are suspected. On the other hand, an exuberant reaction to common pathogens is provided as another speculative ethiopathogenesis for the pyogranulomas. It is feasible that this dog may have been immunosuppressed due to possible hyperadrenocorticism and had severe granulomatous reactions to common pathogens (e.g. botryomycosis). Hyperadrenocorticism however was not definitely proven in this case as the post ACTH cortisol values were within the borderline of 17-22 µg/dl (7). In the case presented here ACTH stimulation was performed rather than a low dose dexamethasone test (LDDST) due to a low specificity of the latter and the risk for false positive results in a very sick dog (7). Unfortunately, the diagnosis of hyperadrenocorticism could not have been confirmed or ruled out on necropsy, since the adrenal and pituitary glands were not examined histopathologically due to an oversight.

Another factor that complicated this case was the limited antibiotic sensitivity of the bacteria that were isolated**.** This could, in part, explain the progression of the initial skin wound which may have then progressed into the generalized disease.

In the case presented here one must note the temporal connection of the original skin wound and the application of the anti-flea product. To the best of the author's knowledge this is the first report that this type of reaction after administration of imidacloprid is described. It is possible that the severe skin and systemic reactions that followed the first skin laceration were caused by the individual sensitivity to the medication (allergic contact dermatitis, irritant contact dermatitis, adverse drug reaction, toxic epidermal necrolysis), or because of other debilitating factors in this specific individual such as immune-suppression or severe bacterial or fungal infections, though none of these factors were proven. Imidacloprid is a neonicotinoid, a class of insecticides that, like nicotine, acts on the nervous system, as an agonist of the nicotinic acetylcholine receptors causing blockage of postsynaptic nicotinergic acetylcholine receptors. The low affinity of neonicotinoids for vertebrate relative to insect nicotinic receptors is a major factor in their favorable toxicological profile (3, 4, 8). If adverse effects are to be expected, the mechanism of action should involve nicotinic receptors and not the development of granulomatous reactions. However, an *in vitro* study on human lymphocytes has shown that imidacloprid can cause DNA damage or genome mutations

at dose levels of 0.05-0.5 mg/L (9). The effect of therapeutic or even higher doses of imidacloprid on inflammatory cells in the skin needs to be investigated. In the human literature there is a description of two fatal cases due to this insecticide. Imidacloprid was identified in the stomach contents of both cases indicating acute imidacloprid intoxication and it was concluded that these two persons committed suicide by ingesting this insecticide. In that report the measurement of imidacloprid and its metabolites in post-mortem samples was described (10). These measurements were not done in the dog reported here as there was no history of ingestion of the product. The fatal human cases should encourage the veterinary clinician to instruct the owners to put the anti-flea product on body areas that the pet can not reach by licking and to prevent other pets from licking the area. Imidacloprid intoxication in this case is unlikely due to the time lag between the application of the product and death and the lack of neurologhical signs. Other causes of death could also not be definitely proven by the post mortem examination, and organ failure due to the systemic pyogranulomas was highly suspected. However, as mentioned previously, a definitive cause for the development of the pyogranulomas has not been established. If the case described here was a result of the application of this product, it was probably also the result of other individual factors as mentioned above and not only the properties of the imidacloprid. A search of the scientific literature has not revealed any publication in which imidacloprid was the cause of this kind of adverse effects. While in this case a severe disease occurred after the administration of imidacloprid, and since the association between the insecticide and the systemic disease cannot be proven, the author still continues to recommend the use of this product in flea control on

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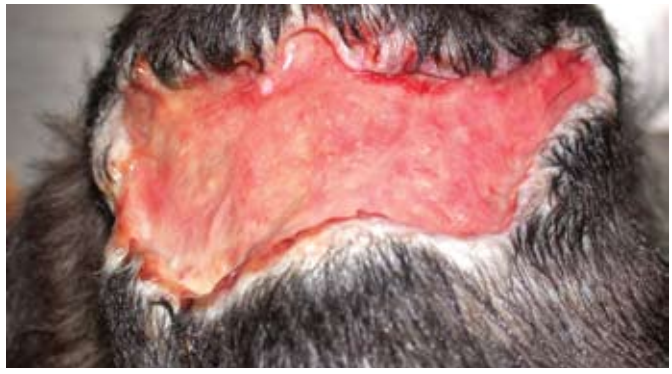

Fig.1 Skin of dorsal neck. Non-healing ulcer

Fig.2 Skin of dorsal neck. Non-healing ulcer

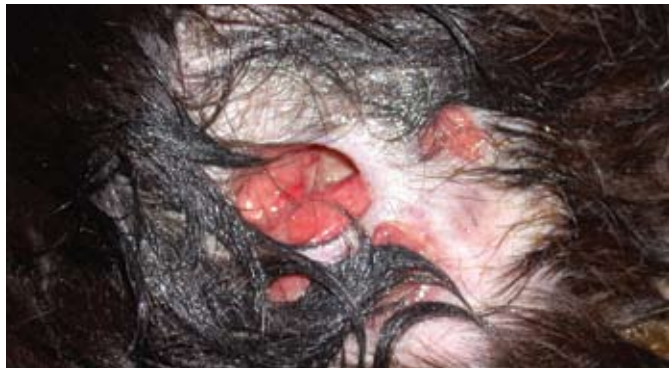

Fig.3 Skin of the back and the head showing erosive lesions

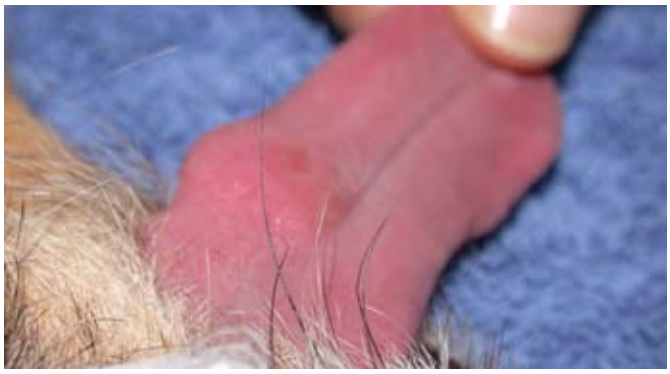

Fig.4a Nodule on the tongue

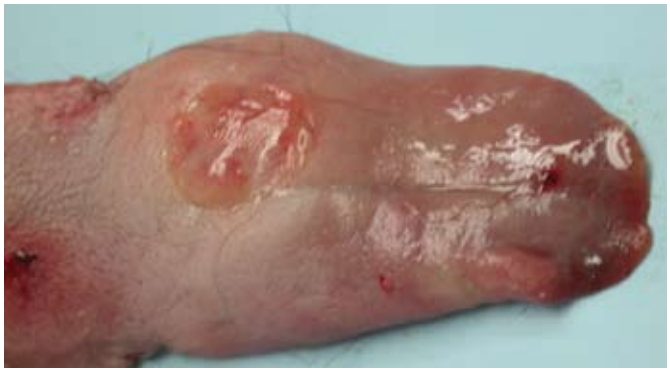

Fig.4b Nodule on the tongue at post mortem

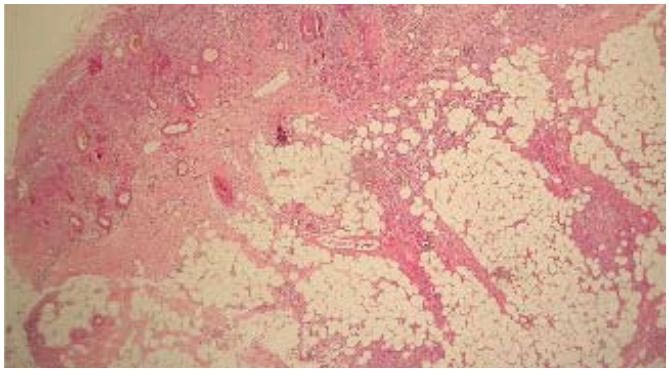

Fig.5 Histopathology of skin showing pyogranulomatous reaction. Original magnification X40 H&E.

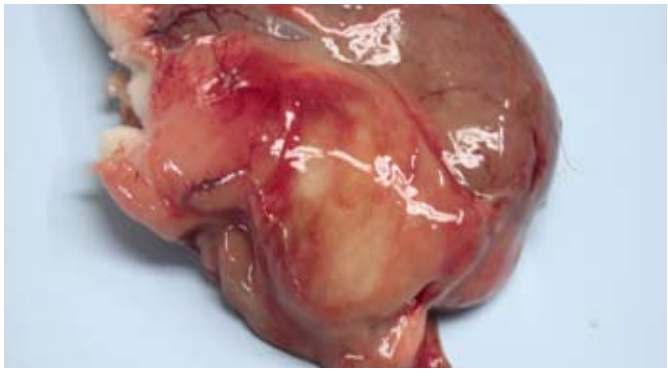

Fig.6 Nodules in the heart

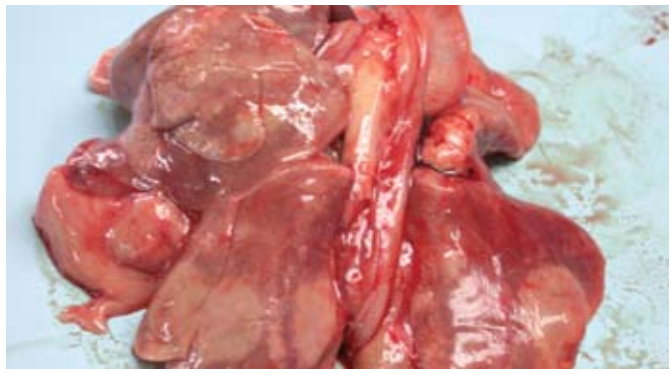

Fig.7 Nodules in lungs

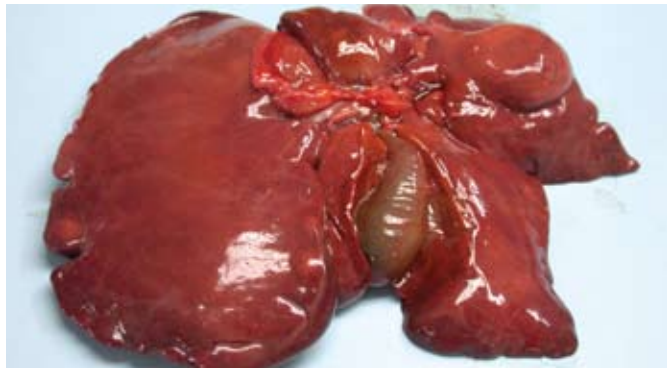

Fig.8 Nodules in liver

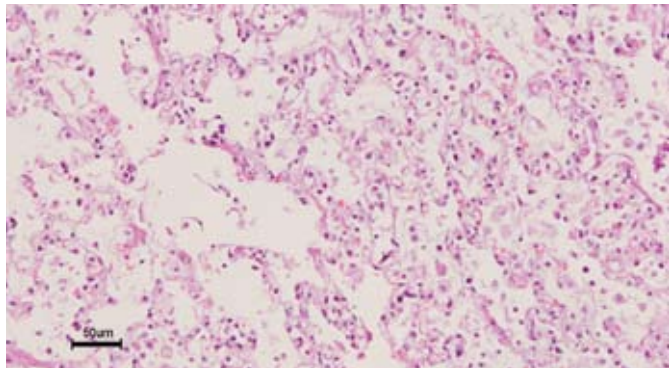

Fig.9 Histopathology of the lungs, showing granulomatous reaction. Original magnification X100, H&E, bar = 50 µ.

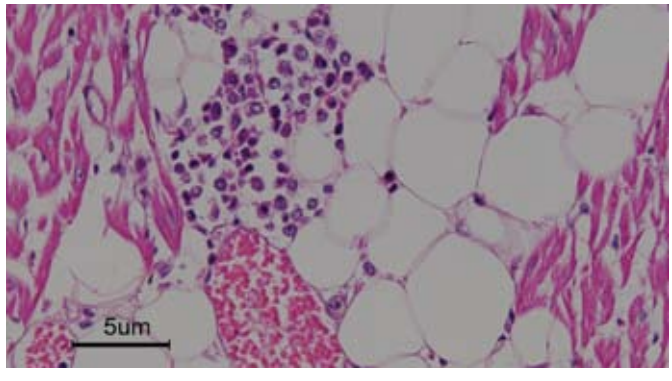

Fig.10 Histopathology of the heart showing granulomatous reaction. Original magnitude X100, H&E, bar = 50 µ.

Table 1. CBC Results on presentation:

Parameter	Value	Reference range
White cell count ($X10^9$/l)	**53.19**	6-17
Neutrophil absolute number ($X10^9$/l)	**45.74**	3-12
Lymphocyte absolute number ($X10^9$/l)	4.25	1-4
Monocytes absolute number ($X10^9$/l)	2.66	0.1-2
Eosinophil absolute number ($X10^9$/l)	0.54	0.2-1.2
Red blood cell count ($X10^{12}$/l)	**5.0**	5.5-8.5
Hemoglobin (g/dl)	**9.5**	12-18
Hematocrit (%)	**28.85**	37-55
Mean corpuscular volume (μ)	**57**	60-77
Mean cell haemoglobin concentration (g/dl)	33.1	31-34
Platelets ($X10^9$/l)	255	200-500

Abnormal values displayed in bold

Table 2. Serum chemistry results on presentation

Parameter	Value	Reference range
Total protein (g/dl)	5.1	5.43- 7.11
Albumin (g/dl)	**1.62**	2.83-3.83
Total bilirubin (mg/dl)	0.33	0.02-0.54
Cholesterol (mg/dl)	**495**	118-309
Triglycerides (mg/dl)	**168**	15-100
Urea (mg/dl)	13.18	11-70
Creatinine (mg/dl)	0.26	0.36-1.36
Aspartate aminotransferase AST (iu/l)	36	9-47
Alanine aminotransferase ALT (iu/l)	18	5-103
Alkaline phosphatase ALP (iu/l)	**970**	4-140
GGT (iu/l)	2	0-19
Calcium total (mg/dl)	**5.07**	8.5-11.0
Phosphorus (mg/dl)	**7.0**	2.63-5.83

Abnormal values displayed in bold