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Eosinophilic Granulomatous Dermatitis Due to Hairy vetch (*Vicia villosa* Roth) Poisoning in a Holstein Cow

Y. Eroksuz^{1*}, E. Polat², C. Akdeniz Incili¹, B. Karabulut¹, A. Sagliyan², H. Eroksuz¹

¹ Department of Pathology, Faculty of Veterinary Medicine, Firat University, Elazig-Turkey

² Department of Surgery, Faculty of Veterinary Medicine, Firat University, Elazig-Turkey

ABSTRACT: Eosinophilic granulomatous dermatitis has been reported due to hairy vetch (*Vicia villosa* Roth) consumption in a 3-year-old Holstein cow. The cow had dermatitis over the face, neck, palpebral skin and perineum in the last 2-months. The other 3 animals also exhibited similar skin lesions on the same farm.

Histological examination of biopsy samples indicated the presence of multifocal eosinophilic granulomatous dermatitis. Immunohistochemical analysis showed that T lymphocytes were the predominant inflammatory cells in granulomatous lesions, whereas B cells and macrophages were less prominent. After the owner stopped feeding cows with the vetch, the skin lesions regressed 6 months later and completely resolved after 12 months. The final diagnosis was hairy vetch poisoning based on plant-lesion relationship, presence of the plant material and character and distribution of the skin lesions and the healing of the lesions after removing the plant from the ration. It is uncommon, but not a rare disease in cattle, however, up to date only one suspected case of hairy vetch poisoning was reported in Turkey.

In conclusion, this report demonstrates that vetch poisoning in cattle could be diagnosed by skin lesions which might also regress by removing vetch from the diet.

Keywords: Bovine; granulomatous dermatitis; hairy vetch (*Vicia villosa* Roth), pathological changes.

Corresponding Author:

Yesari Eroksuz, Department of Pathology, Faculty of Veterinary Medicine, Firat University, Elazig-Turkey
E-mail address: yeroksuz@firat.edu.tr

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INTRODUCTION

Hairy vetch (*Vicia villosa*; *V. villosa*) is an annual or winter annual legume plant with a high-protein value. It is grown for soil improvement, hay, silo feed, or grazing. Despite being so favourable for animal feeding, the consumption of vetch by cattle may result in 3 different clinical syndromes; the first one is a cyanogenic glycoside, the second is a systemic disease with subcutaneous swellings of the head, neck and purulent nasal discharge and bronchitis, and the third one is granulomatous inflammatory changes in various tissues or organs (Mauldin and Peters-Kennedy, 2015; Panciera et al., 1992).

The most common form is the granulomatous disease characterized by the granulomas containing eosinophils, epithelioid macrophages, lymphocytes and plasma cells. These lesions are detectable in the skin, heart muscle, *thyroid*, *liver*, *pancreas*, *mammary glands*, *urinary bladder*, cerebral meninges, kidney, lymph nodes and adrenal glands (Manu M. Sebastian, 2007; Mauldin and Peters-Kennedy, 2015; Fighera and Barros, 2004). Over recent years, a number of studies indicated that *V. villosa* is a toxic plant for cattle in different geographic regions.

However, there are no epidemiological studies investigating the potential effects of hairy vetch consumption on cattle health in Turkey. Further, up to date only one suspected case of hairy vetch poisoning has been reported in Turkey (Oruc et al., 2012).

The present report describes the eosinophilic granulomatous dermatitis due to *V. villosa* poisoning in a 3-year-old Holstein cow.

CASE HISTORY

A 3-year-old, Holstein cow was admitted to the Surgery Clinic of Animal Hospital of Firat University. The cow was from a small family farm having 5 animals including 3 Holstein cows, and two calves. Anamnesis indicated that all the. The owner stated that 3 cows and one calf showed dermatitis over the face, neck and perineum in the last 2-months and he also signed an informed consent form prior to intervention and publication (Protocol No. 200379).

Three cattle were kept in the same barn. Papulonodular skin lesions were present on the right side of the abdomen and medial part of the ventral part of all the cattle and 12-month-old calf. The owner stated that these lesions were pruritic. Four animals were fed with vetch hay, barley hay, concentrated food, and

corn meal for the past 2.5 months. However, no skin lesions were present in the 3-month-old suckling calf. Skin samples were collected by surgical biopsy under local anaesthesia and sedation from one cow.

The skin samples were fixed in 10% formalin, processed routinely, embedded in paraffin, sectioned in 4-5 μ m and stained with hematoxylin-eosin (H&E). Selected tissue sections were stained Brown-Brenn's stain (B&B), periodic acid-Schiff (PAS) and Ziehl-Neelson (Z-N).

Immunohistochemical staining for CD3, CD20 and CD68 were performed by using avidin-biotine complex (ABC) method. Appropriate positive and negative controls were also prepared by using 3, 3' diaminobenzidine as chromogen. The negative controls were processed in the same way, using buffer solution in the state of the primary antibody. The tissue section from a bovine lymph node was used as positive control. Tissue sections are stained on automated stainer (Ventana Medical Systemi Arizona, USA) for CD3 (anti-mouse Dako-M7254, 1:200 dilution) and anti-mouse CD20 (L26, Cell Marque, 1:400 dilution), anti-mouse CD68 (Zeta Clone, KP1, Corporation, 1:200 dilution).

RESULTS

Histopathological changes included focally extensive epithelial necrosis and diffuse parakeratotic hyperkeratosis in the epidermis (Fig-1). In the superficial epidermis, perivascular infiltrations containing lymphohistiocytic and eosinophilic cells were present. There was circumscribed, multifocal to coalescent granulomas including eosinophils, epithelioid macrophages, lymphocytes, multinucleated cells and plasma cells in the dermis. The granulomas ranged from 20 to 100 μ m in diameter. They are usually surrounded by multilayered collagen-containing connective tissue. Immunohistochemistry demonstrated a marked and diffuse and immunoreactivity to CD3, whereas CD68 staining was not so prominent. Rarely B cells (CD20 $^{+}$) were detected in the granuloma. After the owner stopped feeding the vetch, skin lesions regressed 6 months later and completely resolved after 12 months. Neither acid-fast bacteria, nor fungus or parasitic agent was detectable with the special stains.

DISCUSSION

Vicia contains more than 150 species, and 4 of them are known as toxic plants including *V. villosa*, *V. sativa* (Panciera et al., 1992), *V. faba* and *V. angus-*

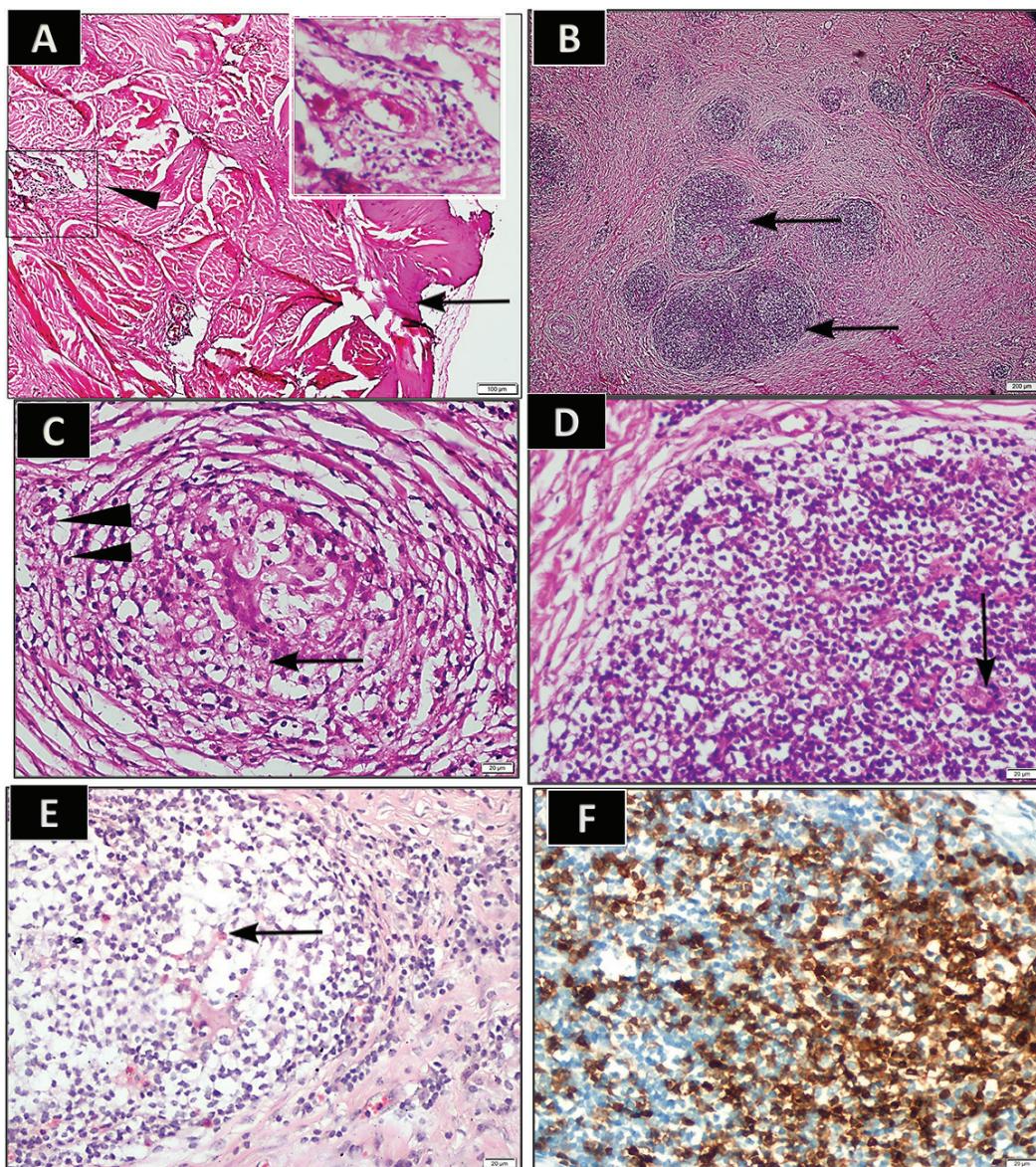


Figure 1: Histopathological and immunohistochemical features of skin lesions. (A–F).

A: Complete epidermal necrosis and diffuse hyperkeratosis (arrow), H&E, x10.

B: Perivascular infiltrate containing lymphocytes, eosinophil leukocytes and a few macrophages in the superficial dermis. H&E, x40.

C: Coalescing and well-demarcated granulomas in the dermis (arrow) H&E, x4.

D and E: The granuloma containing epithelioid cells, eosinophils leucocytes (arrowheads) and multinucleated giant cell (arrow) and fibrous tissue band, H&E, x40.

F: The granulomatous infiltrate containing high numbers of T cells (CD3⁺), x40, ABC method.

tifolia (Barros et al., 2001). Since the first description in USA 5 decades ago (Panciera et al., 1966) in cattle, hairy vetch poisoning has world-wide distribution including England, Wales, France, Brazil, Argentina, South Africa and the Netherlands (Fighera and Barros, 2004; Johnson et al., 1992; Odriozola, et al., 1991; Panciera et al., 1992). Diagnosis depends on history of exposure, clinical signs and pathological changes ((Manu M. Sebastian, 2007, Mauldin and Peters-Kennedy, 2015).

In this report, the final diagnosis was based on

of plant-lesion relationship, the presence of the plant material, and character and distribution of the skin lesions and the healing of the lesions after removing the plant from the ration. The absence of skin lesions in a suckling calf also supports the diagnosis. Holstein breed and older or equal to 3 years cows are reportedly more susceptible (Mauldin and Peters-Kennedy, 2015, Aguirre et al., 2021) which is consistent with our findings.

All the earlier reports and classical veterinary pathology textbooks showed the multi-organ involve-

ment in hairy vetch poisoning in cattle. However, skin involvement is the most common and severe as compared to other tissues. As shown in previous publications, eosinophils are always one of the cell components in granulomas.

There is no scientific consensus on the etiology of granulomatous inflammation in vetch poisoning. However, the hypothesized chemical agents are: prussic acid (Hargis and Ginn 2012), canavanine (Prete, 1985), cyanamide (Kamo et al., 2003) and lectins (Panciera et al., 1992). Similar to the previous finding (Panciera et al. 1992), T lymphocytes were the predominant inflammatory cells in granulomatous lesions in this report. Direct activation of T lymphocytes reportedly stimulates cytotoxicity and granulomatous inflammation which qualities of a type-IV hypersensitivity reaction (Panciera et al., 1992). Dermatitis begins with alopecia and later exudation and pruritis occur.

Differential diagnosis includes citrus pulp toxicosis, sylade poisoning, diureido-isobutane (Matsukawa et al., 1983) and vetch-like disease (pyrexia/pruritis/hemorrhagic syndrome) (Panciera et al., 1992; Saunders et al., 2000). In Turkey, the hairy vetch is widely cultivated and included in the rations of cattle. As a result, potential new cases of poisoning might occur in cattle in future unless the farmers are informed.

Taken together collectively, this report demonstrates that the occurrence of vetch poisoning in cattle in Turkey and skin lesions are highly diagnostic of hairy vetch toxicity and they might regress by removing vetch from the diet.

AUTHORS' CONTRIBUTIONS

PE and SA carried out surgical biopsy and macroscopical description. EY, EH, KB, CAI performed histopathological and immunohistochemical examinations. EY and EH wrote the draft the manuscript. We confirm that the manuscript has been read and approved by all named authors

DECLARATION OF CONFLICTING INTERESTS

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. There has been no significant financial support for this work.

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