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CASE REPORT

Suspected Hairy Vetch (*Vicia villosa*) Poisoning with a Concomitant Babesiosis in Dairy Cows in Turkey

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ABSTRACT

Nine Holstein cows were died between 3 October and 13 December of 2009 in Turkey. On this farm, all the dairy cattle (350) were being fed in two groups by the owner. The only difference between the rations was that the ration of group 2 was containing hairy vetch hay. It was noticed that all (except one dead) animal were in the Group 2. Clinical signs in affected animals prior to death were generally dramatic drop in milk yield, inconsistent fever, diarrhea, cutaneous lesions, listless, ruminal stasis, conjunctivitis, icterus, red urine and abortion in the pregnant animal. Anemia and *Babesia bigemina* were detected in hematologic analysis. There was disseminated vacuolar degeneration around vena centralis in liver, and the epithelium of the proximal tubules had severe swelling and had deeply eosinophilic epithelial cells and the lumens of tubules were disappeared in kidneys. It is considered that the cases presented here are mainly caused by hairy vetch as no more clinical signs and deaths were seen after 10 days of removal of hairy vetch from the animals' ration.

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INTRODUCTION

Hairy vetch (*Vicia villosa*) is a legume that grows throughout the United States and in other temperate climates of the world (Panciera *et al*., 1992). Hairy vetch poisoning occurs most often when it forms a major part of diet of cattle and horses, and when the plant is near maturity (Knight and Walter, 2001). The plant is less likely to cause a problem in hay or when ensiled (Panciera *et al*., 1992). The causative factor(s) of vetch-associated disease are not known (Fighera and Barros, 2004). However, hairy vetch contains the toxic arginine analogue, canavanine, and hairy vetch seeds also contain lectins, canavanine and γ-Glutamyl-S-Ethenyl-Cysteine (GEC) (Enneking, 1995). The generalized granulomatous disease that is characteristic of hairy vetch poisoning in cattle and horses is suggestive of hypersensitivity (type IV) reaction induced by a foreign substance that activates the immune system response (Harper *et al*., 1993). Hairy vetch poisoning is prevalent and more severe in cattle over three years of age (Knight and Walter, 2001). Although reported in many breeds of cattle, hairy vetch poisoning appears to be more common in the Angus and Holstein breeds (Fighera and Barros, 2004).

Hairy vetch poisoning sporadically affects adult cattle and is characterized by signs of pruritic dermatitis, weight loss, conjunctivitis and diarrhea (Knight and Walter, 2001), and fever (Odriozola *et al*., 1991; Fighera and Barros, 2004). Abortions (Johnson *et al*., 1992) and red urine (Panciera *et al*., 1992) have also been associated with hairy vetch poisoning. Subcutenous swelling, ulcers of the oral mucous membranes, and purulent nasal discharges with significant mortality may also occur (Fighera and Barros, 2004).

Hairy vetch poisoning has been reported from cattle associated with grazing hairy vetch in United States (Johnson *et al*., 1992), Australia (Harper *et al*., 1993) and Argentina (Odriozola *et al*., 1991). In the literature, to our best knowledge we could not find any report about hairy vetch hay poisoning in cattle in intensive feeding condition, which is based on zero-grazing, and with babesiosis. In addition, this is the first report on hairy vetch poisoning in Turkey. Therefore, this paper reports the epidemiology, clinical signs and pathology of hairy

vetch poisoning with babesiosis in a Holstein cattle herd in intensive feeding condition.

History, clinical examination & postmortem findings: Nine Holstein cattle (eight dairy cows and one pregnant cow), between 3.5 and 4 years old, died on a cattle farm in Bursa, Turkey between 3 October and 13 December (from middle of autumn to beginning of winter in Turkey), 2009. The farm consists of 350 cows kept on intensive feeding systems in two groups. Eight animals were died in group 2 out of 150 cows, in which a ration containing hairy vetch hay, dairy feed, wheat straw, alfalfa hay, pasture hay, and corn silage were fed to the animals. The animals in group 1 (200 cows) were fed with same ration like that of group 2 except hairy vetch hay, and only one cow died during this period of time. Each animal in group 2 received 1.5-2 kg hairy vetch hay daily. The cases occurred from 2 to 4 months after the animals were started feeding with hairy vetch hay ration.

Clinical symptoms were seen in affected cows 1-5 days prior to the death. Two cows were found as died in their barn. Cases were noticed firstly when the milk yield of the affected animals was dramatically dropped between 50 and 80%. Clinical findings were generally inconsistent fever that varied from 39.5 to 41°C, diarrhea, weakness, ruminal stasis, conjunctivitis and red urine. Blood smears of all affected animals were stained with Giemsa and *Babesia bigemina* was detected**.** In addition, hematologic analysis showed that red blood cell (between 3.20 are 4.10 Mil/µl) and hemoglobin levels (between 6.70 and 7.4g/dl) were decreased. Cutaneous lesions were seen generally on head and neck. Abortion was seen in an 8 months pregnant cow that was found died from group 2 and the animal had two fetuses.

Postmortem examination was firstly made by the attending veterinarian in nine dead animals and in one of the pregnant cow's fetuses. Gross lesions were generally observed in spleen, kidneys, liver and lymph nodules. Affected spleen, liver, gall bladder, kidneys and lymph nodules were generally enlarged. Two of the spleens were ruptured. The livers had accentuated lobular pattern and they were generally icteric. The kidneys were also slightly icteric. There was a pattern of systemic lesions consist of multifocal to coalescing gray-white soft to moderately firm nodules infiltrated particularly in the cut surface of spleen and kidney. In addition, a layer that in gray to white in color was noticed on the skin, hair and lips of two fetuses (Fig. 1) which we herein report these findings for the first time. Subsequently, liver, spleen and kidneys of the pregnant cow and one of her fetuses were submitted to the Pathology Department for pathological examinations.

Microscopically, the covering layer that in gray to white in color on fetuses was generally consisting of squamous epithelium cells**.** In liver, there was disseminated vacuolar degeneration around vena centralis and also seen bile stasis in the bile canaliculi of all lobules, and fibrin deposition, Kuppfer cell activation with mononuclear cell infiltration was noticed in sinusoids (Fig. 2). Megacaryocytes were seen in white pulp of the spleen (extra medullar hematopoiesis). Fibrine exudation, eritrophages and siderocyte were also seen in the spleen which was hemorrhagic. In kidneys, the epithelium of the proximal tubules had severe swelling and had deeply eosinophilic (nephrosis/necrosis) epithelial cells and the lumens of tubules were disappeared. The glomeruli had swelled and filled Bowman space. Edema was seen in intertubular areas (Fig. 3).

Fig. 1: A layer that is gray to white in color on skin and hair of the fetus

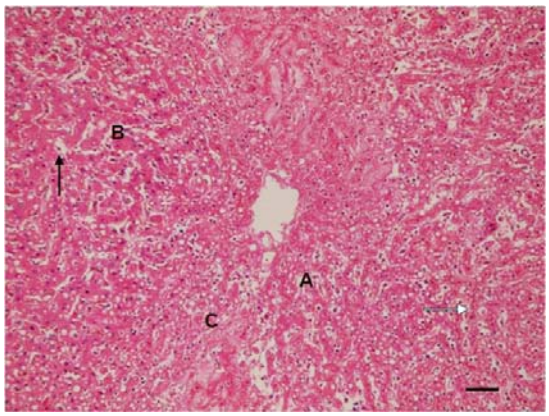

Fig. 2: Vacoulization (A) around the vena centralis in liver, stagnation in bile canaliculi (open arrow); dilatation in sinusoids (B), fibrine deposition (C), activation of Kuppfer cells (black arrow). H & E, 100 µm.

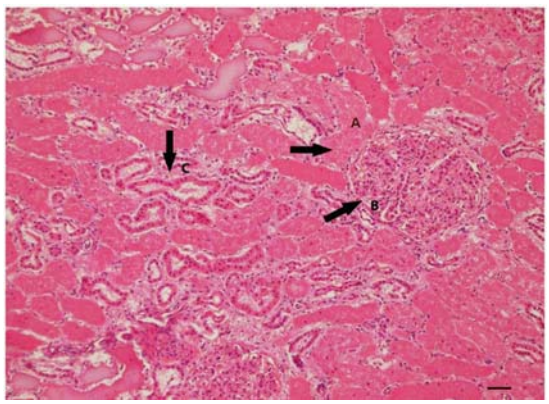

Fig. 3: Swelling in tubule epithelium, increased eosinophilic stained (nephrosis/necrosis) in cytoplasm (A), swelled and filled Bowman space (B), edema in intertubular areas (C) in kidney. H & E, 100 µm.

Diagnosis: Although some of the clinical symptoms were harmonious with babesiosis such as fever, anemia, red urine, icterus and diarrhea (also generally seen these symptoms in hairy vetch poisoning); some symptoms and necropsy findings were different such as dermatitis, gray-white firm nodules which infiltrated especially spleen and kidneys. In addition, eight animals out of nine died animals

were being fed with hairy vetch hay ration. Consequently, it was suspected that the vetch had been purchased mistakenly as *Vicia pannonica* by the farm owner. So, the vetch samples were sent for identification to the Biology Department of Uludag University. Indeed, the samples were not *V. pannonica* and they were identified as *Vicia villosa*. On the other hand, blood smears of all affected animals were stained with Giemsa and *B. bigemina* was detected**.** In addition, hematologic assays showed that red blood cell (between 3.20 are 4.10 Mil/µl) and hemoglobin levels (between 6.70 and 7.4g/dl) were decreased.

DISCUSSION

It has been reported (Panciera *et al*., 1992) that an Angus cow which was being fed with a 9 kg of hairy vetch (wet weight) daily got sick on day 11 and died on day 24. In our case, each animal in group 2 was being fed with 1.5-2 kg hairy vetch hay (dry matter) daily, so lower amount of hairy vetch hay consumption for 2-4 months might have caused the poisoning, The epidemiological data and the clinical necropsy, and histopathological findings observed in died cows indicate hairy vetch poisoning (Harper *et al*., 1993; Knight and Walter, 2001).

The specific toxin(s) in hairy vetch responsible for the symptoms encountered in hairy vetch poisoning has not been determined (Panciera *et al*., 1992; Fighera and Barros, 2004). Some of the clinical signs observed in affected animals are associated with babesiosis which is a common disease in this area. Although clinical babesiosis is usually seen in summer months in Turkey, a recent work has reported on clinical babesiosis occurred in cattle in February (winter month in Turkey) (Kar *et al*., 2008). The authors found only on one and three animals with high and no parasitaemia ticks of the genus *Boophilus annulatus*, respectively. In our case, ticks could be detected neither on the affected animals nor inside the barn. The attending veterinarian noticed that anaplasmosis was observed in cattle in last summer on this farm. From this point of view, it seems likely that the animals were exposed to tick infestations and become infected subclinically with babesiosis (may be in the summer months) prior to the onset of clinical cases. In the present case, the clinical signs related to hairy vetch poisoning and babesiosis observed in dead animals were generally from group 2 animals (eight cows) that were feeding with hairy vetch hay ration. Thus, these clinical signs may have arisen as a result of the suppressor effects of hairy vetch (canavanine) on the immune system of affected animals (Morimota *et al*., 1990; Enneking, 1995).

The findings seen in the pregnant animal was consistent with severe hemolysis, hemorrhage-siderosis in spleen, icterus and siderosis in liver; nephrosis and necrosis in kidney. The cause of death of this animal and its fetus may be related with anoxia. To our best knowledge, this is the first report on hairy vetch poisoning with a concomitant babesiosis in cattle in intensive feeding conditions where also an unusual layer that in gray to white in color was detected on the skin, hair and lips of the aborted fetuses (Fig. 1).

In conclusion, case history, clinical findings, necropsy and histopathological findings obtained from the dead animals point out hairy vetch poisoning. Since no more deaths occurred 10 days after removing the hairy vetch hay from the ration of group 2, hairy vetch hay consumption could be the main reason for deaths in this case.

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