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# Concurrent multi-systemic eosinophilic epitheliotropic disease and choledocholithiasis in a 15-year-old mixed-breed mare

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## Abstract

A mixed-breed 15-year-old mare was euthanized for recurrent colic, severe weight loss, pastern ulcerative dermatitis, and coronitis without response to non-surgical medical procedures. The liver was congested with firm consistency at necropsy, and there were multi-focal white spots on Glisson's capsule. Hemorrhage, ulcer, severe folding, and hypertrophy were seen in the duodenum and jejunum. Histopathological examination showed marked infiltration of eosinophils concomitant with fewer leukocytes, macrophages, and plasma cells, primarily in the liver and intestines. The presence of eight calcium bilirubinate stones, 2.00 - 5.00 cm in diameter, in the common bile duct was characteristic of choledocholithiasis. Concurrent multi-systemic eosinophilic epitheliotropic disease (MEED) and choledocholithiasis were confirmed after ruling out other potential causes of eosinophilic infiltration, including parasitic infection and allergy. The cause of MEED and choledocholithiasis was unclear because of incomplete case history and delayed referral, with a potential infection playing a role and having synergistic effects between the two conditions. When eosinophilic-associated inflammation involves several organs, MEED should be on the differential diagnostic list since infections and allergens are also among the risk factors for this disorder.



## Introduction

Multi-systemic eosinophilic epitheliotropic disease (MEED) is a rare chronic disease distinguished by eosinophils and lymphoplasmacytic cells infiltrating multiple organs. It is one of the inflammatory bowel diseases, typically found in young Standardbred and Thoroughbred horses.[1,2] This condition can affect the integumentary system, gastrointestinal tract, liver, pancreas, and, rarely, the lungs. It leads mainly to skin disorders, severe weight loss, recurrent colic, and diarrhea.[3,4] On the contrary, choledocholithiasis, characterized by the calculi (gallstones) in the common bile duct, is a prevalent disorder in elderly equines.[5,6] These calculi can cause biliary obstruction and hepatic diseases; however, they are sometimes detected during necropsy without prior clinical signs of hepatic damage.[1,2,6]

Type I hypersensitivity reactions to parasites or insect bites, inflammatory responses to antigens and tumors, and autoimmunity are believed to lead to eosinophil infiltration and MEED in animals.[2,3] The cause of cholelith formation is unknown, but cholangiohepatitis, foreign body, bile stasis, and changes in bile composition may be contributing factors. Previous infections may also play a role in both conditions, but are not always present at diagnosis.[5,6]

This report outlines the clinical signs and diagnostic findings of a unique case of a 15-year-old mixed-breed mare with concurrent MEED and choledocholithiasis.

## Case Description

**Clinical examination.** A 15-year-old mixed-breed mare had undergone treatment for incurable coronitis, pastern lesions, recurrent colic, and severe weight loss for over a year. The mare was referred for euthanasia due to the severity of the conditions. The animal appeared cachectic with a body score of one and had a rough and unkempt hair coat (Fig. 1A). The mare had alopecia, old scars, crusting on several parts of the body, an ulcer on the left side of the vulva, conjunctival hyperemia, and ocular purulent discharge in the left eye. The owner believed that the old scars, pastern ulcerative dermatitis, and coronitis were caused by trauma.

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The rectal temperature was 35.80 ˚C, with a respiratory rate of 16 breaths *per* min and a resting heart rate of 32 beats *per* min. Loud intestinal borborygmi sounds were heard on the right flank, but were less detectable on the left side. During rectal palpation 2 days before euthanasia, a large volume of pellet-shaped feces, multiple intestinal loops, and many nodules on the intestinal walls were detected. The animal had a poor prognosis after a year of unsuccessful treatment and was referred for euthanasia by the equestrian club. The subject animal was sedated using xylazine (Rooyan Darou, Semnan, Iran) administered intravenously at a dosage of 0.50 mg $kg^{-1}$ of body weight. Following the onset of recumbency, a saturated magnesium sulfate solution (Scharlab, Barcelona, Spain) was administered intravenously at a dosage of 2.00 mL $kg^{-1}$ until euthanasia was confirmed.

**Clinical pathology and microbiology.** Complete blood count with differential and plasma protein tests showed no significant abnormalities. The serum gamma-glutamyl transferase (GGT) was measured as 47.00 U $L^{-1}$ (normal range: 4.00 - 44.00). This measurement was conducted using an enzymatic colorimetric assay following the International Federation of Clinical Chemistry (IFCC) method, with absorbance assessed at 405 nm (bichromatic 405/600 nm) at a controlled temperature of 37.00 ˚C. Additionally, the total bilirubin level increased to 54.70 µmol $L^{-1}$ (normal range: 17.00 - 35.00) with a direct bilirubin of 7.50 µmol $L^{-1}$ (normal range: 0.00 - 6.80). These measurements were obtained using a colorimetric diazo method, with spectrophotometric analysis conducted at wavelengths between 540 - 570 nm, also at 37.00 ˚C. All assays for GGT and bilirubin levels were performed utilizing commercial reagent kits from Pars Azmoon Co. (Tehran, Iran). The examination of the peritoneal fluid by a binocular microscope (Zeiss 20 Standard; Carl Zeiss AG, Oberkochen, Germany) showed a noteworthy infiltration of eosinophils and neutrophils (Fig. 1B); no specific pathogens were identified during multiple cultures. The total bilirubin of the peritoneal fluid was 14.40 µmol $L^{-1}$ (normal range: 8.00 ± 6.20), with a direct bilirubin level of 1.00 µmol $L^{-1}$. During two sampling periods 2 days before euthanasia, the parasitic fecal egg count was negative.

**Gross pathology.** Serous atrophy and orange-yellow serofibrinous exudate in the abdominal cavity were the first findings during the necropsy. The kidneys and lungs were hyperemic, and several dark mediastinal lymph nodes were observed. The spleen appeared pale with rounded edges and many petechiae on its capsule. The liver was congested and had rounded borders, a firm consistency, and a red-to-green-brown discoloration. In addition, multi-focal white spots were present on the Glisson's capsule, which contained parasite migration pathways. The common bile duct was dilated and contained eight yellow-green calculi (choledocholithiasis) concentric in cut and 2.00 - 5.00 cm in diameter. They had a stiff consistency and rough surface (Fig. 1C) and were later identified as calcium bilirubinate stones with a 75.00% mixture of cholesterol and bilirubin, 20.00% calcium oxalate, and 5.00% phosphate. Mesenteric lymph nodes were enlarged and firm, whereas intestinal walls were moderately hypertrophic, edematous, mildly icteric, and hyperemic, with bulging veins. Hemorrhage, ulceration, severe folding, and hypertrophy were observed in the duodenum and jejunum (Fig. 1D). The cecal wall revealed severe hyperemia and petechiae.

**Histopathology.** Tubulointerstitial nephritis was observed in the kidneys, with tubular coagulative necrosis and peripheral glomerular fibrosis. In addition, lymphocytic mononuclear inflammatory cells and a few eosinophils infiltrated the interstitial tissue. The lungs were affected by moderate pulmonary edema, hyperemia, and interstitial pneumonia. The lymph nodes exhibited lymphadenitis, follicular and sinus hyperplasia, mild eosinophil infiltration, brown stains, and probably hemosiderin. The spleen was involved with hemosiderosis, hemorrhage, and atherosclerosis.

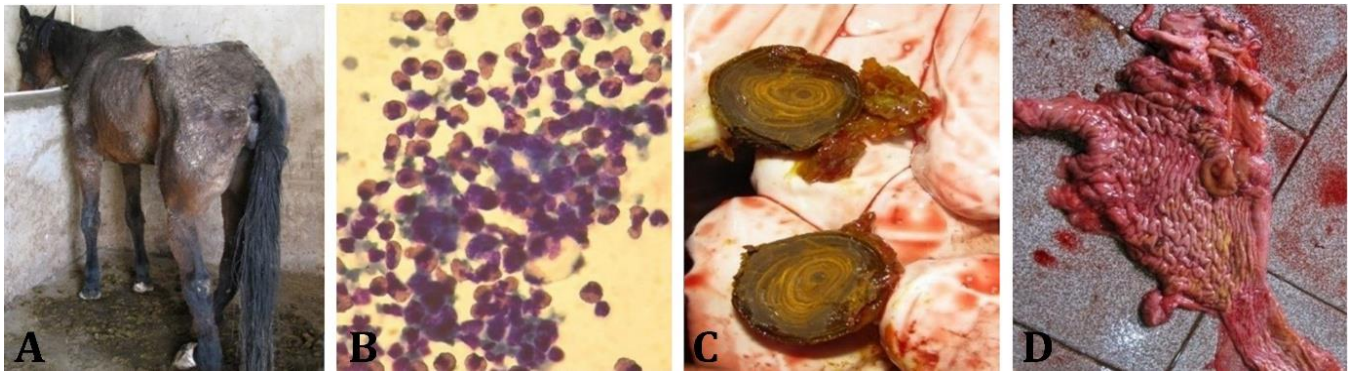


**Fig. 1.** Clinical and gross pathology findings. **A)** The severely emaciated 15-year-old mixed-breed mare referred for euthanasia; **B)** The direct smear of peritoneal fluid with severe eosinophilic and neutrophilic infiltration (oil objective lens, N. A.=1.25, 100 ×); **C)** Choledocholithiasis, yellow-green calcium bilirubinate stones with a markedly laminated appearance; **D)** The lumen of the duodenum and jejunum with hemorrhage, ulcer, severe folding, and hypertrophy.

Histopathological evaluation by the binocular microscope of the liver revealed severe eosinophilic infiltration in the peri-portal and intra-lobular spaces and severe atherosclerosis. Hepatocytes in the peri-portal ducts were moderately enlarged with pale cytoplasm and contained bile pigments surrounded by macrophages and lymphocytes. The liver exhibited hemosiderosis, hyperplastic biliary ducts with bile retention, and extensive hepatocyte necrosis that resulted in liver fibrosis. Degenerated eosinophils, macrophages, brown pigment, inflammatory infiltrates of lymphocytes, and plasma cells were found in several necrotic granulomatous areas surrounded by Langhans giant cells (Figs. 2A and 2B). Ulcers, crypt necrosis, villous atrophy, and fibrinous secretions were observed mainly in the duodenum and jejunum. Severe eosinophilic enteritis was characterized by the widespread infiltration of eosinophilic cells and fewer lymphocytes, plasma cells, and macrophages in the intestinal lamina propria and submucosa (Figs. 2C and 2D).

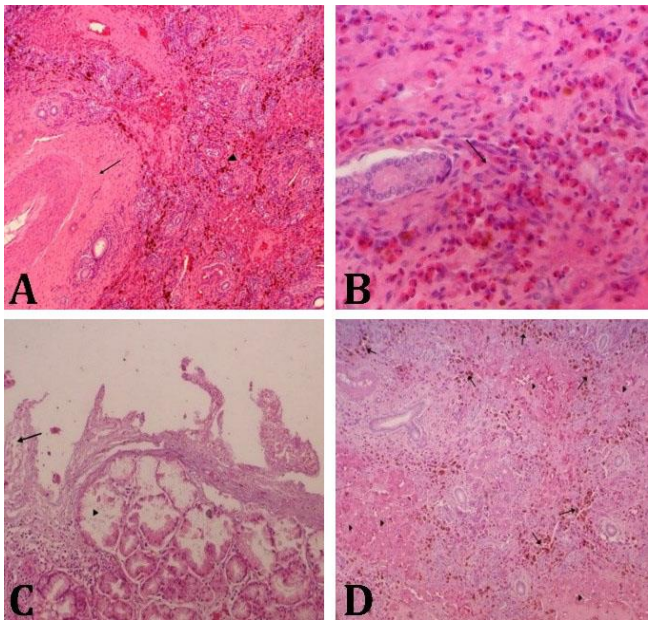


**Fig. 2.** Histopathological findings. **A)** atherosclerosis (arrow), infiltration of eosinophils and congestion in the liver (arrowhead), (Hematoxylin and Eosin staining, 40×); **B)** Hepatonecrosis, connective tissue replacing normal parenchymal tissue, and predominant eosinophils infiltration (arrow), (Hematoxylin and Eosin staining, 100×); **C)** Superficial necrosis of the small intestine (arrow) associated with congestion and submucosal edema (arrowhead), (Hematoxylin and Eosin staining, 40×); **D)** Eosinophilic enteritis, and multi-focal infiltration of eosinophils, leukocytes, and macrophages in mucosa and submucosa (arrows), along with fibrin deposition (arrowheads), (Hematoxylin and Eosin staining, 40×).

## Discussion

Since 1982, a few cases of MEED have been published worldwide. It is commonly found in young Standardbreds and Thoroughbreds.[1,4] This makes the current mixed-breed equine, aged 15, a rare case and the first one from Iran. This case was also unique because the combination of MEED and choledocholithiasis had not been reported before. Any infection could have triggered both and may have had a synergistic effect or pathophysiological interaction.

In the present case, the diagnosis of MEED was made as other causes of eosinophil infiltration were not confirmed, such as parasitic infection and allergy.[7] Choledocholithiasis may be an incidental finding during necropsy. Even though choledocholithiasis can increase the possibility of cholestasis, severe fibrosis, and related clinical manifestations, the increases in biochemical elements did not confirm the bile stasis here.[8,9] A multi-fold increase in serum GGT is expected in MEED and cholelithiasis.[4,9] However, the GGT increase in the current case was also insignificant.

The complications of MEED are usually attributed to hyperplasia, fibrosis, and numerous granulomas rather than eosinophilic infiltration.[1,2,4] Numerous granulomas with eosinophil infiltration were evident in the liver. These granulomas may have a background of parasites, but no parasites were detected in the sections. Fibrosis around the bile ducts can also signify bile retention;[5] however, severe fibrosis can be related to both problems in the current case. Like most reported MEED, eosinophilia and neutrophilia were not observed in this case.[4] However, intensive infiltration of eosinophils and neutrophils in the peritoneal fluid may indicate active inflammation, particularly when the bile ducts are disrupted.[7,9]

Intermittent fevers in MEED and choleliths have been repeatedly reported due to the liver involvement.[4,8] Still, it was inconsistent with our findings. As the respiratory system can be involved in MEED,[1,2] interstitial pneumonia and atherosclerosis were noted here, but eosinophilic infiltration was insignificant. Lymph nodes are usually involved in MEED, consistent with our findings.[3] Severe multi-tissue atherosclerosis was observed in the present case, as vascular involvement in MEED has been previously reported.[1]

The prognosis of MEED is poor, and treatments, such as corticosteroids, temporarily stabilize the condition.[1,2,7] Choledocholithiasis can be diagnosed using ultrasound and removed with surgery.[8] Restoring hepatic function is possible depending on the location of the stones, severity of liver tissue involvement, disease stage, and role of racial diversity in liver regeneration.[8] However, the prognosis of the current case would still be poor due to the MEED.

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## Conflict of interest

The authors state that they have no financial or personal relationships that could influence the case reported in this paper.

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