Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

CASE REPORT

Scrotal Hydrocele in a Dog with Dirofilaria Infestation and Cholangiocellular Carcinoma

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ARTICLE HISTORY

Received: December 21, 2010
Revised: May 03, 2011
Accepted: May 04, 2011

Key words:
Dirofilaria
Dog
Hydrocele
Hypoproteinemia
Liver
Scrotum

ABSTRACT

A four-year-old male Saint Bernard dog with a scrotal hydrocele was referred with a history of scrotal swelling and emaciation. Physical and hematological evaluation revealed dirofilaria infestation and liver function abnormalities. Ultrasonography showed fluid collection in each peritesticular area and in the peritoneal cavity. The dog survived only 10 days after the initial presentation. At necropsy, umbilicated nodular masses in the liver, hemorrhagic ascites, heart dirofilariasis, and accumulated transudate in the scrotum were observed. Histopathologic and immunofluorescence examination revealed cholangiocarcinoma in the liver, indicating the cause of liver failure and ascites accumulation. Severe edema was seen in the mediastinal connective tissue of spermatic cord and heartworm DNA from the spermatic cord tissue was detected by polymerase chain reaction (PCR) analysis. In the present case, it was suspected that the acquired hydrocele might have been caused by ectopic migration of filarial worms or by severe hypoproteinemia induced by liver failure.

To Cite This Article: Yu DH, YS Roh, H Park, GH Woo, S Ejaz, K Lee, CW Lim, J Park and B Kim, 2011. Scrotal hydrocele in a dog with dirofilaria infestation and cholangiocellular carcinoma. Pak Vet J, 31(4): 375-378.

INTRODUCTION

A hydrocele is a collection of serous fluid within the layers of the tunica vaginalis of the scrotum. Two different types of scrotal hydroceles have been reported: communicating and non-communicating. A communicating hydrocele occurs because of an opening in the tunica vaginalis that allows abdominal fluid to communicate between the scrotum and body cavity (Hsu *et al*., 2004). Non-communicating hydroceles are congenital with spontaneous recovery in infants (Christensen *et al*., 2006) and secondarily caused in adults (Abdel-Rheem, 1983). The causes of such acquired scrotal hydroceles are obscure, but inflammatory responses, neoplasm in the testis and epididymis, or testicular torsion can lead to the development of this condition. Lymphatic obstruction by filarial worms and lymphatic hypoplasia are also important etiologies in some cases (Dandapat *et al*., 1986). Others have suggested that scrotal hydroceles may be caused by an inadequate balance of exudation and absorption of fluid in the tunica vaginalis due to increased capillary permeability and a lymphatic defect (Abdel-Rheem, 1983), or secondarily due to severe ascites (Abbitt *et al*., 1995; Hsu *et al*., 2004). Accordingly, hydroceles are now classified into several types based on the cause, but idiopathic scrotal hydroceles are also widely reported in humans.

Dirofilaria infections are common in dogs worldwide. The adult worms are usually found in the right ventricle and pulmonary arteries, but some dirofilariasis occurs in unusual locations, such as the spermatic cord, epididymis, liver, subcutaneous tissue, abdominal cavity, breast, and conjunctival tissue. In humans, filarial or microfilarial infestation is closely related to lymphatic obstruction and microfilaremic hydroceles in men (Lammie *et al*., 1993), but no such case or study has been reported in animals. This case describes a scrotal hydrocele in a dog with dirofilaria infestation and severe hypoproteinemia caused by hepatic failure.

History and Clinical Examination

A four-year-old male Saint Bernard dog referred to the Animal Medical Center, Chonbuk National University, Korea, presented with a pendulous swelling in

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the scrotal region and a history of chronic weight loss. Clinical examination of the dog revealed bilateral scrotal swelling (Fig. 1a) with anorexia. Physical examination also showed abdominal distension, pale and icteric mucous membranes, and mild fever (40.6°C). Hematologic analysis revealed neutrophilic leukocytosis and a non-regenerative anemia (HCT 28.7%). Serum biochemical analysis showed a marked decrease in serum albumin (1.5 g/dl) and cholesterol level (80 mg/dl). Hyperbilirubinemia (1.9 mg/dl) and increased gamma-glutamyltransferase (11 U/L) and alkaline phosphatase (154 U/L) were also noted, and fasting serum bile acid concentrations (104 μM, reference range 0~25 μM) were remarkably elevated, indicating hepatic failure. In addition, a commercial ELISA kit (SNAP 3Dx, IDEXX, USA) for heartworm antigen detection showed a positive result and microfilaria were further observed on blood smear examination. Subsequent urinalysis showed bilirubinuria. A real-time, ultrasonic scanning of the scrotum failed to reveal any structural abnormalities such as an inguinal hernia or testicular torsion, but a large amount of peritesticular fluid was visible around each testicle (Fig. 1b). In addition, diffuse hyperechoic hepatic parenchymal alterations, a thickened gall bladder wall, and ascitic fluid was observed on ultrasonogram. A scrotal hydrocele with concurrent hepatic failure and dirofilariasis was occasionally diagnosed, but the prognosis was poor and the survival time was 10 days after diagnosis.

Postmortem findings

On postmortem examination, widespread distributions of many white and umbilicated nodular masses of varying sizes were observed in all lobes of the liver (Fig. 2a). The masses were well demarcated from the surrounding liver tissue. Large numbers of filarial worms were found in the right side of the heart (Fig. 2b) and in the hepatic vein. The cut surface of the scrotal wall was thick and edematous, and the extratesticular fluid was pure transudate. A large amount of hemorrhagic ascites was present in the peritoneal cavity (Fig. 2c).

Tissue samples from the organs, including the liver and testis with the spermatic cord, were collected, fixed in 10% neutral phosphate-buffered formalin, and processed by standard histologic paraffin methods. Tissue sectioned at 5 μm was stained with hematoxylin and eosin (H&E) and examined by light microscopy. The tumor mass and nodules were composed of neoplastic bile duct epithelial cells with tubular structures, indicating cholangiocarcinoma (Fig. 2d). Confocal microscopic examination revealed that the tubular structures were positive for carcinoembryonic antigen (Fig. 2e), which is specifically expressed in the bile duct cells and components of cholangiocarcinoma. Severe edema was observed in the mediastinal connective tissue of the spermatic cord (Fig. 2f), but inflammation was not observed in the tissue. Obstruction of the lymphatics with microfilaria was not detected in serially sectioned spermatic cord tissue. A conventional PCR assay was conducted using "pan-filarial primers" designed to amplify different fragment lengths from *D. immitis* and *Acanthocheilonema (Dipetalonema) reconditum* as previously described (Rishniw *et al*., 2006) (Fig. 3). Sequence analysis of the 536-bp fragments showed that this species had a 98% homology with *Dirofilaria immitis* from Chongqing in China (GenBank No. EU182331).

DISCUSSION

Ultimately, we diagnosed the dog with an acquired scrotal hydrocele induced by dirofilaria infestation and by severe hypoproteinemia in liver cancer. Acquired hydroceles have mostly been reported in humans (Gyapong *et al*., 1998), and various studies, including analysis of protein content in hydrocele fluid (Witte *et al*., 1973), lymphography (McBrien *et al*., 1972), and clearance rate of injected dyes (Abdel-Rheem, 1983), have been conducted in human cases. Although such intensive examinations have not been performed in the current study, detection of filarial DNA in the severely edematous mediastinal connective tissue of the spermatic cord was demonstrated in the dog, suspecting that circulatory disturbance as a possible cause of the scrotal hydrocele might be caused by filarial obstruction.

Filarial vessel or lymphatic obstruction could have induced the hindrance of protein in the tunica vaginalis, and fluid accumulation might have been accelerated by the increased osmotic pressure, since hypoproteinemia was noticeable in the dog, along with hepatic failure. However, direct evidence for lymphatic obstruction could not be confirmed by histopathology in the serially sectioned spermatic cord. It was difficult to confirm whether filarial nematode obstructed lymphatic vessels since most of connective tissues were severely edematous and many lymphatic vessels were collapsed by severe edema. In addition, inflammation and fibrosis in the spermatic cord tissue, which are usually observed in the obstructed lymphatic vessel during filarial infestation in human, were not observed in the spermatic cord tissue. This finding suggests that the scrotal hydrocele in the present case might have been influenced by other factors. Ascites induced by hepatic failure could be another cause of communicating scrotal hydrocele (Hsu *et al*., 2004; Abbitt *et al*., 1995). At necropsy, hemorrhagic ascites was evident in the peritoneum, but accumulated fluid in the extratesticular lesion was not hematoceles, indicating no communication of fluid between the peritoneum and scrotum. Trauma, infection to the scrotum, or inguinal herniation are other potential causes of acquired scrotal hydrocele, but the dog had no such history before the development of the hydrocele, and several diagnostic investigations revealed the absence of an inguinoscrotal hernia or inflammation with pyoceles in the scrotum. Accordingly, such possible factors for the development of hydrocele were excluded in the present case.

Detection of the filarial antigen and antibody in the hydrocele fluid represent another method to diagnose obstructive hydrocele. A study of 100 human patients with scrotal hydrocele suggested a close correlation between serum positivity for filarial antigen/antibody and hydrocele (Goel *et al*., 2006). Such testing was not performed in the present study, but PCR detection of the filarial antigen in the tissue involved in the hydrocele, as used in our study, may offer a practical and alternative method for diagnosing acquired scrotal hydrocele induced by filarial infestation.

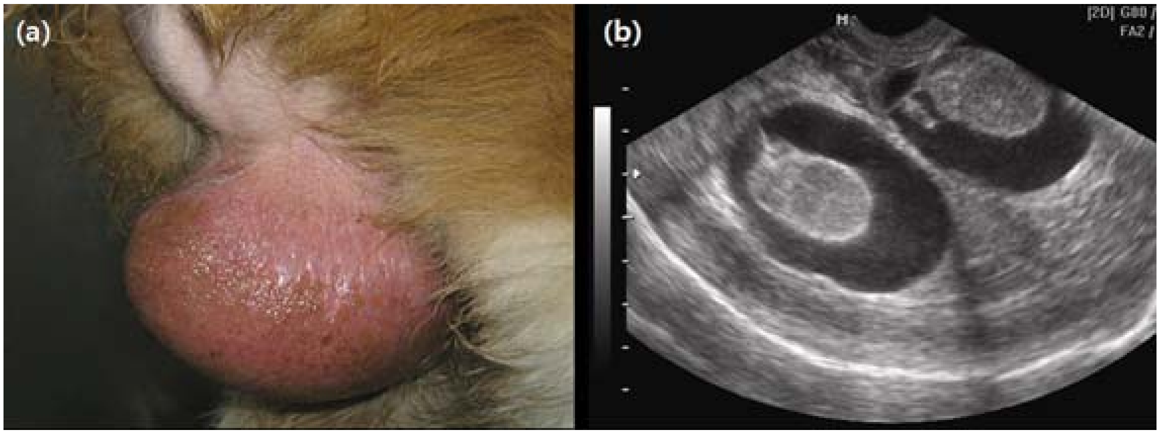

Fig. 1: Scrotal hydrocele in a dog. (a) The pendulous and congested scrotum was bilaterally swollen. (b) A real-time ultrasonic scanning image gives a picture of scrotal tissue with a large amount of hydrocele fluid in the peritesticular space.

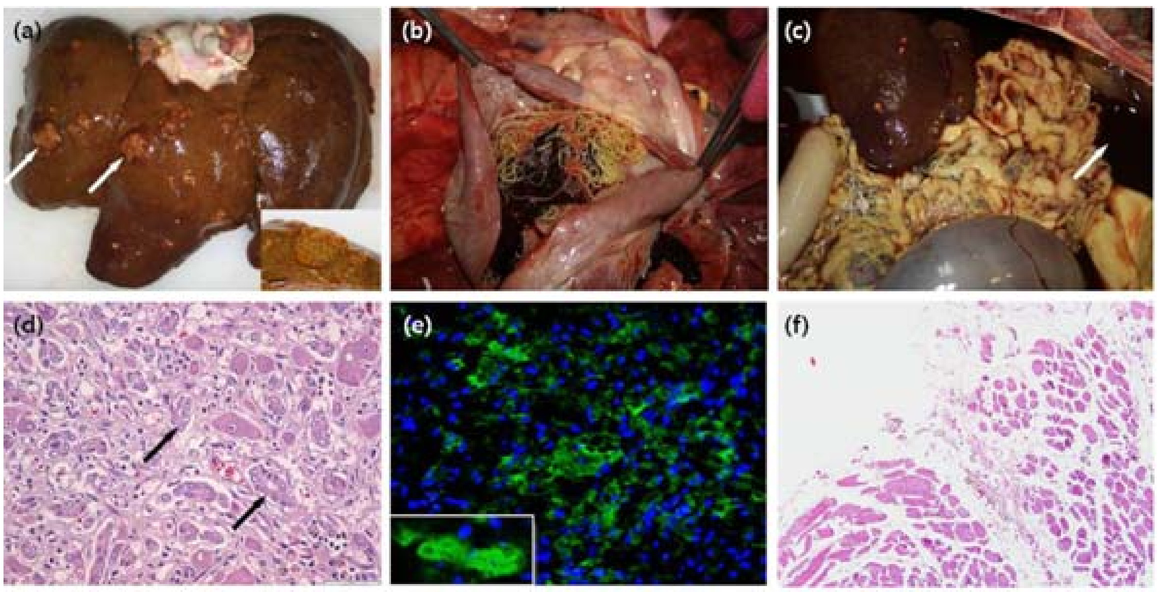

Fig. 2: Postmortem examination and histological appearance of the liver and testicular tissue. (a) Nodular masses of various sizes (arrows) were found in all hepatic lobes, and the masses were well-demarcated from the surrounding liver tissue (insert). (b) Large numbers of heartworms were present in the right ventricle of the heart. (c) Hemorrhagic ascites (arrow) were observed. (d) Tumor tissue with organizing, neoplastic, bile duct epithelial cells (arrows) with tubular structures (H&E stain, ×400). (e) Immunofluorescence reactivity for bile duct type carcinoembryonic antigen was massively identified in the tumor tissue, and tubular/glandular structures were noted (insert) (FITC anti-human CD66 antigen staining method, ×400). (f) Severe edema in the mediastinal connective tissues of spermatic cord is seen (H&E, ×200).

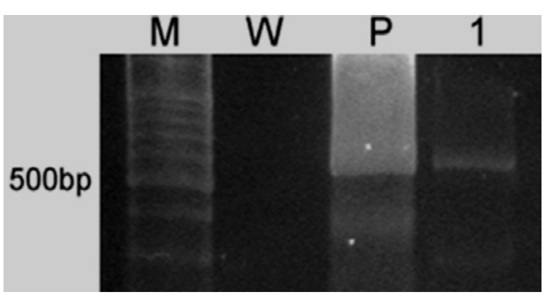

Fig. 3: Detection of *D. immitis* in the testicular tissue of a dog. The arrow indicates the position of the *D. immitis*-specific PCR product. M: molecular size marker (1K-bp DNA Ladder); P: positive control; W: reagent negative control (water); 1: tissue sample.

Acknowledgments

This study was supported by National Research Foundation of Korea Grant funded by the Korean Government (2009-0064313).

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