

A Case of Persistent Müllerian Duct Syndrome with Sertoli Cell Tumor and Hydrometra in a dog

Aya MATSUU^{1)*}, Takuya HASHIZUME¹⁾, Teppei KANDA¹⁾, Masashi NAGANO²⁾, Akihiko SUGIYAMA³⁾, Yoshiharu OKAMOTO⁴⁾ and Yoshiaki HIKASA⁵⁾

¹⁾Departments of Veterinary Internal Medicine, ²⁾Theriogenology, ³⁾Laboratory Medicine, ⁴⁾Veterinary Neurology and Oncology and ⁵⁾Veterinary Internal Medicine, Faculty of Agriculture, Tottori University, Koyama-Minami 4-101, Tottori 680-8553, Japan

(Received 13 May 2008/Accepted 7 November 2008)

ABSTRACT. A 10-year-old Miniature Schnauzer with bilateral cryptorchidism and male external genitalia was referred with a history of abdominal enlargement. Upon exploratory laparotomy, two tumors and a connecting structure similar to fluid-filled uterus were recognized. After cytological and bacterial examinations of the fluid and histological examination, this dog was diagnosed with bilateral Sertoli cell tumor with hydrometra. The karyotype of this dog was 78, XY and the *sry* gene was detected positive by PCR. We diagnosed this dog as a case of persistent Müllerian duct syndrome (PMDS), which is male pseudohermaphroditism. This is the first report regarding the incidence of PMDS in Miniature Schnauzers in Japan, and it suggests the involvement of a gene carrier.

KEY WORDS: hydrometra, Miniature Schnauzer, persistent Müllerian duct syndrome.

J. Vet. Med. Sci. 71(3): 379–381, 2009

A 10-year-old male Miniature Schnauzer was referred to Tottori University Animal Teaching Hospital with a history of abdominal enlargement. Physical examination revealed bilateral cryptorchidism with male external genitalia and non-pruritic dorsal lumbar alopecia. On palpation, a large mass was felt in the abdomen. The general condition seemed to be normal, and visible feminization was not recognized.

Full CBC and serum biochemistry tests were performed. Slight elevations were observed in the levels of alanine amino transferase (182 U/l), alkaline phosphatase (1,363 U/l), and total cholesterol (259 mg/dl). The concentrations of serum estradiol and testosterone were 103 pg/ml (reference range of normal male: < 15 pg/ml) and 246 ng/dl (reference range of normal male: 100–300 ng/dl), respectively. Abdominal radiographs detected large masses compressing the normal intestinal canal. Abdominal ultrasonography revealed two masses with mixed echogenicity. Additionally, hypoechogenicity tubular cysts cranial to the bladder were observed, which appeared to be fluid-filled like pyometra or hydrometra.

Although the tubular cysts were unidentifiable, the abovementioned findings suggested the possibility of testicular tumors; thus, exploratory laparotomy was performed to explore the possibility of resecting the masses and cysts. A large and a small mass, which were speculated to represent the testicles, were located at the center of the abdomen and attached to the structure like the uterus which was inflated with fluid material. This uterus-like structure had two horns that caudally joined into the body of the uterus, the vagina terminated cranially into the prostate gland. Due to the

growing mass, obvious structures like the fallopian duct or the testicular canal were not recognized. After the vagina was ligated, both the tumor and the uterus were extracted (Fig 1). Cytodiagnosis revealed intact erythrocyte and keratinizing epitheliums without pathogenic microorganisms. To identify the presence of aerobic bacteria, culture was performed and any multiplication of bacteria were not observed. The walls of both the uterine horns were thin. Histological examination confirmed bilateral Sertoli cell tumor and normal uterus; no ovarian tissue was found. The postoperative general condition was good; there is no evidence of metastasis so far. Alopecia in the dorsal lumbar region improved 124 days after surgery.

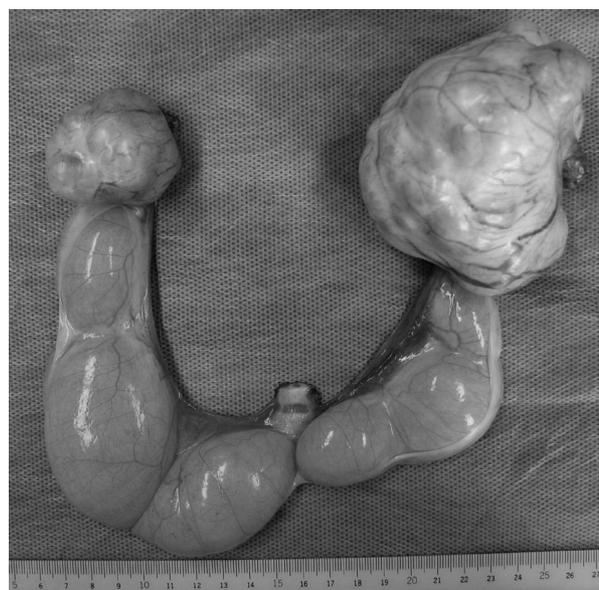


Fig. 1. Gross appearance of the extracted tumors and the uterus.

* CORRESPONDENCE TO: MATSUU, A., Department of Veterinary Internal Medicine, Faculty of Agriculture, Tottori University, Koyama-Minami 4-101, Tottori 680-8553, Japan.
e-mail: matsuu@muses.tottori-u.ac.jp

To investigate the karyotype of the dog, chromosome preparations were obtained from a peripheral blood lymphocyte culture and stained with Giemsa [4]. Cytogenetic evaluation revealed a normal male chromosome complement, 78, XY.

In order to amplify the fragment of *sry* gene (*sry*), genomic DNA was isolated from the blood samples obtained from the dog by using a genomic DNA extraction kit (GFX Genomic Blood Purification Kit, GE Healthcare, Buckinghamshire, UK); concurrent samples were obtained from a normal male beagle and a female beagle as positive and negative controls. The primer pair was used according to the previous report [6] as follows: Forward: 5'-CTC GCG ATC AAA GGC GCA AGA T-3', Reverse: 5'-TCC GGC TTC TGT AAG CAT TTT C-3'. Polymerase chain reaction (PCR) was performed on 25 μ l of a mixture containing 1 μ g of template genomic DNA, 10 pmol of each primer, 200 μ M deoxynucleoside triphosphate (dNTP), and 1.25 units Taq DNA polymerase (TaKaRa, Shiga, Japan). The PCR was repeated for 35 cycles with denaturation for 40 s at 94°C, annealing for 40 s 60°C, and extension for 1 min at 72°C to obtain a 104-bp fragment. The PCR product was separated by electrophoresis using 2.0% agarose gel with TBE buffer, stained with ethidium bromide, and photographed in ultraviolet light. According to the results, the dog was *sry* positive (Fig 2).

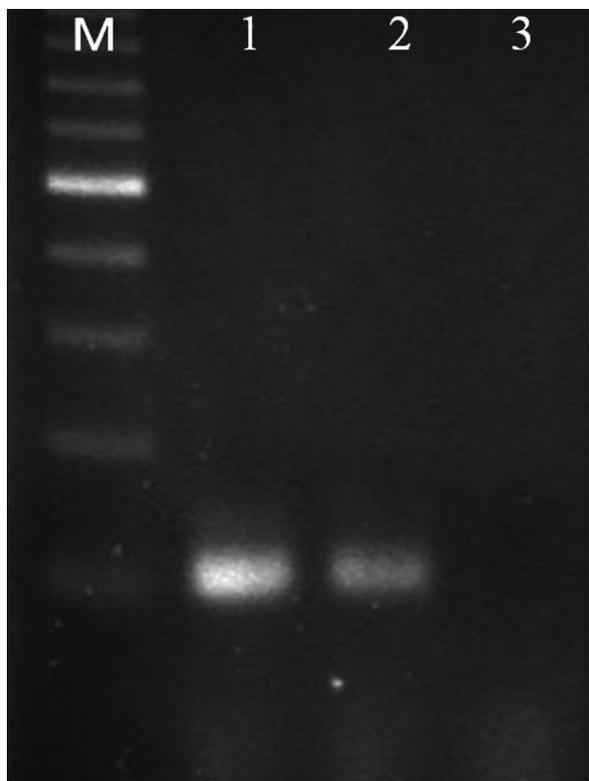


Fig. 2. PCR amplification of *sry*. Lane M, molecular size marker; Lane 1, case dog; Lane 2, male dog; Lane 3, female dog.

Intersexuality is a disorder that is caused by any abnormality during sex determination or differentiation, thereby leading to defects in the reproductive tract. Identification of the male chromosome complement and the presence of the *sry* gene indicate that the dog represents a case of male pseudohermaphroditism [2]. Male pseudohermaphroditism is characterized by XY chromosomal constitution, testes, and feminization of the internal or external genitalia feminized to some extent; this is rarely observed disorder in dogs. This disorder mainly includes persistent Müllerian duct syndrome (PMDS) and androgen insensitivity syndrome. PMDS is a rare form of male pseudohermaphroditism, which is a disorder in the müllerian duct derivatives including the fallopian tubes and uterus [2]. In humans, it is known that this failure is caused by mutations of the müllerian inhibiting substance (MIS) gene, which is produced by sertoli cells of the fetal testis and responsible for the regression of müllerian ducts in male fetuses, or the MIS receptor gene [1]. Canine PMDS has been shown to be an inherited autosomal recessive pattern in the experimental familial strain [3]. At certain time periods during embryonic development, affected dogs secrete bioactive MIS, which suggested that the defect in PMDS-affected animals is insensitivity of the müllerian ducts to MIS possibly due to a defect in the MIS receptor [3]. However, the molecular mechanisms and a useful marker of canine PMDS have not been identified until today.

PMDS may be differentiated by clinical signs from complicated diseases like pyometra, torsion of uterus, and testicular tumor [5, 7]; it may also be differentiated by means of the abdominal operation for the treatment of other diseases. In human PMDS, the most common complicated disease is inguinal hernia [1]. The patients may not be recognized throughout their lifetime in the absence of any symptoms. In our case, testicular tumor with cryptorchidism and hydrometra were recognized as complications. Although the accurate mechanisms underlying the occurrence of hydrometra have not been elucidated, it might result from the development of endometrial hyperplasia or an obstruction of the lumen of the uterus, cervix, or vagina [8]. In the case of the present report involving the dog, the müllerian duct could have developed from the uterine, cervix, and upper part of the vagina, which was terminated without opening to female external genitalia including vaginal vestibule and vulva, thereby resulting in the obstruction of the lumen fluid. High concentration of serum estrogen resulted from the celtri cell tumor might have resulted in the formation of the fluid pool.

The incidence rate of this syndrome has not been obvious. Familial PMDS in Miniature Schnauzers has been recognized in the United States [3]; however, there has been no report about this syndrome in Japan. Since dogs with this syndrome experience reproductive failure, further investigation is necessary to identify the gene-carrier dogs.

REFERENCES

1. Josso, N., Belville, C., Clemente, N. and Picard, J. Y. 2005. AMH and AMH receptor defects in persistent Müllerian duct syndrome. *Hum. Reprod. Update* **11**: 351–356.
2. Lyle, S. K. 2007. Disorders of sexual development in the dog and cat. *Theriogenology* **68**: 338–343.
3. Meyers-Wallen, V. N., Donahoe, P. K., Ueno, S., Manganaro, T. F. and Patterson, D. F. 1989. Müllerian inhibiting substance is present in testes of dogs with persistent müllerian duct syndrome. *Biol. Reprod.* **41**: 881–888.
4. Mayers-Wallen, V. N., Palmer, V. L., Acland, G. M. and Her-shfield, B. 1995. Sry-negative XX sex reversal in the American cocker spaniel dog. *Mol. Reprod. Dev.* **41**: 300–305.
5. Myerscough, N. 1993. Pyometra and torsion of the uterus in a male dog. *Vet. Rec.* **4**: 252.
6. Nowacka-Wozzuk, J., Nizanski, W., Klimowicz, M. and Switonski, M. 2007. Normal male chromosome complement and a lack of the SRY and SOX9 gene mutations in a male pseudohermaphrodite dog. *Anim. Reprod. Sci.* **98**: 371–376.
7. Salkin, M. S. 1978. Pyometra in a male pseudohermaphrodite dog. *J. Am. Vet. Med. Assoc.* **15**: 913.
8. Smith, F.O. 2006. Canine pyometra. *Theriogenology* **66**: 610–612.