NOTE *Pathology*

Common variable immune deficiency in a Pomeranian with *Pneumocystis carinii* pneumonia

Hideyuki KANEMOTO[1)]*, Rei MORIKAWA[2)], James Kenn CHAMBERS[2)], Koichi KASAHARA[1)], Yasuko HANAFUSA[3)], Kazuyuki UCHIDA[2)], Koichi OHNO[1)] and Hiroyuki NAKAYAMA[2)]

[1)]*Department of Veterinary Internal Medicine, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo 113–8657, Japan*
[2)]*Department of Veterinary Pathology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo 113–8657, Japan*
[3)]*National Institute of Animal Health, Department of Infectious Disease, Ibaraki, Japan*
[4)]*Department of Animal Radiology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo 113–8657, Japan*

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ABSTRACT. A Pomeranian dog, 1 year- and 8 month-old neutered female, was presented with persistent respiratory distress and recurrent generalized demodicosis. Physical examination revealed cyanosis, rough respiratory sounds, multifocal alopecia and dermal erosions on the dorsal side of the forelimbs, perineal area and skin around the eyes. A severe diffuse interstitial lung pattern was observed on thoracic radiographs. The blood examination revealed neutrophilia and hypoglobulinemia. Serum immunoglobulin concentrations of IgG and IgA were low. Histopathological examination revealed severe diffuse interstitial pneumonia with *Pneumocystis carinii* infection. Severe lymphoid depletion was observed in the spleen and other organs with lymphoid follicles consisted mainly of CD3-positive T cells and few cells of B-cell lineage. B-cell hypoplasia with subsequent antibody deficiency was suspected.
KEY WORDS: B-cell hypoplasia, common variable immune deficiency, *Pneumocystis carinii*

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Species of the genus *Pneumocystis* exists as fungal pathogens that cause opportunistic infection and subsequent pneumonia in various species including human [1, 4, 10, 12, 13, 15, 16, 21]. Several reports described naturally infected dogs, some of which showed concurrent immunodeficiency [2, 8, 11, 17, 19]. Here, we report a clinical and pathological description of a Pomeranian with *pneumocystis* pneumonia, demodicosis and underlying immunodeficiency. Sequence analysis of mitochondrial large-subunit RNA and subsequent phylogenetic tree analysis was also performed. This is the first report of *Pneumocystis* pneumonia in Pomeranians, and the autopsy findings let us to a new insight into the pathogenesis of *Pneumocystis* pneumonia in dogs.

A 20-month-old, neutered, female Pomeranian dog was referred to the Veterinary Medical Center of the University of Tokyo for respiratory distress and coughing, which failed to respond to treatment with antimicrobials for 1 month. The dog also had a history of recurrent generalized demodicosis since her birth. Owing to the severe respiratory signs, her appetite and activity had decreased to 10–20% of her usual healthy condition.

Physical examination revealed severe respiratory distress with panting and cyanotic mucous membranes (arterial oxygen saturation was approximately 70% when breathing room air). The patient's body temperature (38.5°C) and heart rate (120 beats per min) were within the respective reference ranges. The dorsal side of both forelimbs and the perineal area were alopecic, and the skin around the eyes had also developed alopecia and erosions.

Thoracic radiographs showed a severe diffuse interstitial lung pattern with a mild peripheral alveolar lung pattern in focal areas. The main laboratory findings included hypoproteinemia (4.4 g/d*l*) due to hypoglobulinemia (1.4 g/d*l*), leucocytosis (31,700/μl) with neutrophilia (24,400/μl) and monocytosis (5,700/μl) and an increased C-reactive protein concentration (9.0 mg/d*l*; reference range [RR], <1 mg/d*l*). The absolute number of lymphocytes was within the normal range (1,270/μl). No infectious agents were noted during examination of the skin. Serum immunoglobulin concentrations of IgG and IgM determined by the immunodiffusion method were 3.2 mg/m*l* (RR, 8.0–16.0 mg/m*l*) and 1 mg/m*l* (RR, 0.5–2.0 mg/m*l*), respectively; the IgA concentration determined with an enzyme-linked immunosorbent assay (ELISA) was 1.1 mg/m*l* (RR, 1.33–3.14 mg/m*l*). Infectious pneumonia with an underlying immunodeficiency disorder was suspected, and the dog was hospitalized to provide intensive treatment, including oxygen supplementation and antibiotics (Imipenem/Cilastatin (TIENAM; Merck & Co., Inc., Whitehouse Station, N.J., U.S.A.) 10 mg/kg three times a day and sulfadiazine/trimethoprim (Tribrissen Injection, Kyoritsu, Tokyo, Japan) 25 mg/kg once a day). The patient showed no improvement in respiratory state and died on the third day after being admitted to the hospital.

*CORRESPONDENCE TO: KANEMOTO, H., Department of Veterinary Internal Medicine, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1–1–1, Yayoi, Bunkyo-ku, Tokyo 113–8657, Japan. e-mail: akanemot@mail.ecc.u-tokyo.ac.jp

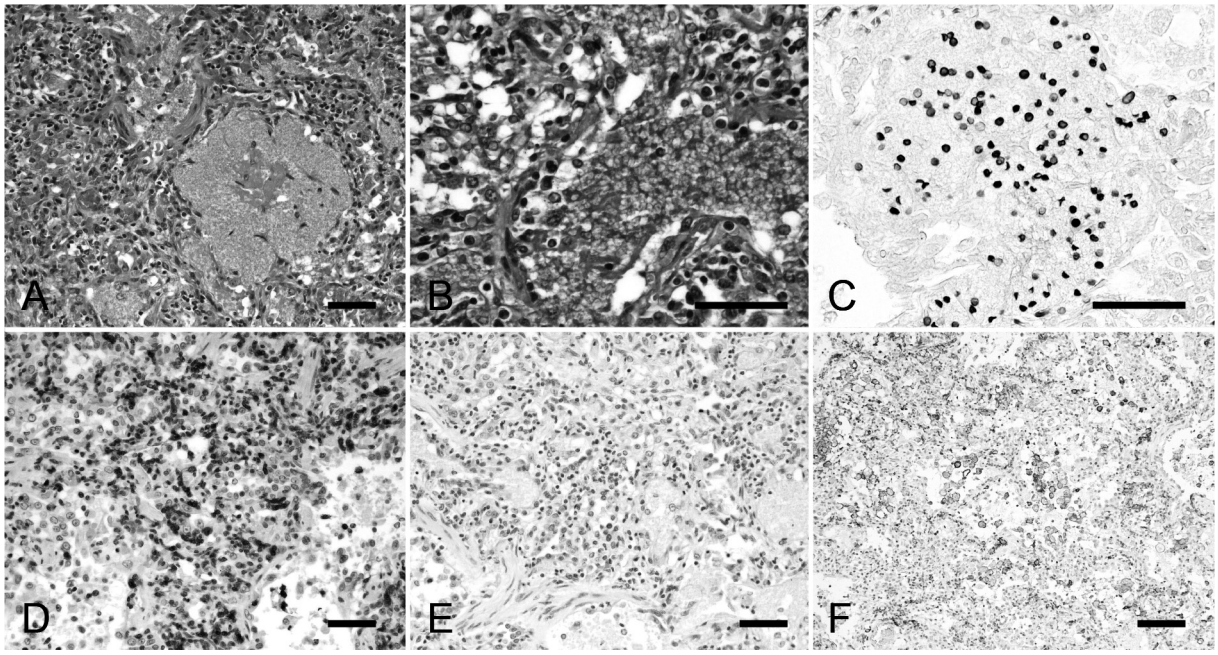

Fig. 1. Hematoxylin and eosin (A), periodic-acid Schiff (B), Grocott (C), anti-CD3 (D), anti-CD20 (E) and anti-Iba-1 (F) immunostaining of the lung. Bar=100 mm. On Hematoxylin and eosin staining, foamy macrophages and small yeast-like organisms filled alveoli and bronchial space with interstitial infiltration of lymphocytes and mild fibrosis. The organisms were positive for periodic-acid Schiff (B) and Grocott (C) stains. Almost all of the infiltrated lymphocytes were positive for anti-CD3 immunostaining (D), and CD20 positive cell was hardly detected (E). Iba-1 positive macrophages were abundant (F).

At necropsy, the lungs were consolidated and weighed 96 g (patient's body weight, 2.4 kg) with diffuse congestion in all lobes. The superficial lymph nodes (superficial cervical, axillary and inguinal lymph nodes) were atrophic and difficult to detect. Histopathological examination revealed interstitial pneumonia characterized by moderate infiltration of lymphocytes, diffuse mild fibrosis and congestion in the alveolar wall. Foamy macrophages and small yeast-like organisms filled the alveolar and bronchial spaces. The organisms were positive for periodic acid-Schiff (PAS) and Grocott's methanamine silver (GMS) stains (Fig. 1A–1C). *Demodex* mites were identified in the hair follicles and were accompanied by a mild infiltration of lymphocytes. The femoral bone marrow was hyperplastic in myeloid and erythroid lineage. Lymphoid cells were euplastic. In the spleen, lymphoid follicle-like structures were observed, but mature germinal centers were absent (Fig. 2A).

Immunohistochemistry was done by polymer enhancing system (Envision, Dako, Tokyo, Japan). The details of antibodies are summarized in Table 1. DNA was extracted from a paraffin-embedded block of lung tissue using the commercially available TaKaRa DEXPAT™ Easy kit (Takara Bio Inc., Tokyo, Japan), and PCR was conducted with primers targeting the mitochondrial large-subunit ribosomal RNA (mtLSU rRNA) genes of *Pneumocystis carinii* (pAZ102-H and pAZ102-E) with GeneAmp® PCR System 2700 (Applied Biosystems, Singapore). An amplicon of approximately 350 bp in size was confirmed with agarose gel electrophoresis. Direct sequence analysis of the PCR product was performed with a DNA sequencing kit (BigDye® Terminator v3.1 Cycle Sequencing Kit, Applied Biosystems, Foster City, CA, U.S.A.), sequenced by a sequencer (3,130 × l Genetic Analyzer, Applied Biosystems, Hitachi, Japan) and analyzed in Neighbor Joining method using MEGA 6.0 [18].

Immunohistochemistry for CD20, IgG and PAX5 [22] revealed a markedly decreased number of B lymphocytes and plasma cells in the spleen, lungs, intestine and skin, whereas CD3-positive lymphocytes were abundant in these tissues (Figs. 1D, 1E, 2C, 2D and 2F–2H). In the bone marrow, both B and T cells were not abundant, but were present in a distribution similar to normal dogs. Anti-ionized calcium binding adaptor molecule 1 (anti-Iba-1) immunostaining showed severe infiltration with macrophages in the lungs (Fig. 1F) and a lack of dendritic cells, which should normally compose the germinal centers (Fig. 2F).

The phylogenetic analysis inferred from the mtLSU rRNA sequence comparison demonstrated that *P. carinii* from dog was different from all previously published *P. carinii* sequences (Fig. 3) and this phylogenetic tree also indicating that dog-derived *P. carinii* is most closely related to *P. carinii* from ferret (*Mustela putorius furo*). The obtained 329 base pairs of nucleotide sequences were deposited in DNA Data Bank of Japan (DDBJ) under accession number LC009003.

This case report describes a Pomeranian with primary immunodeficiency and *P. carinii* pneumonia, which has been reported in miniature dachshunds [9, 11], Cavalier King

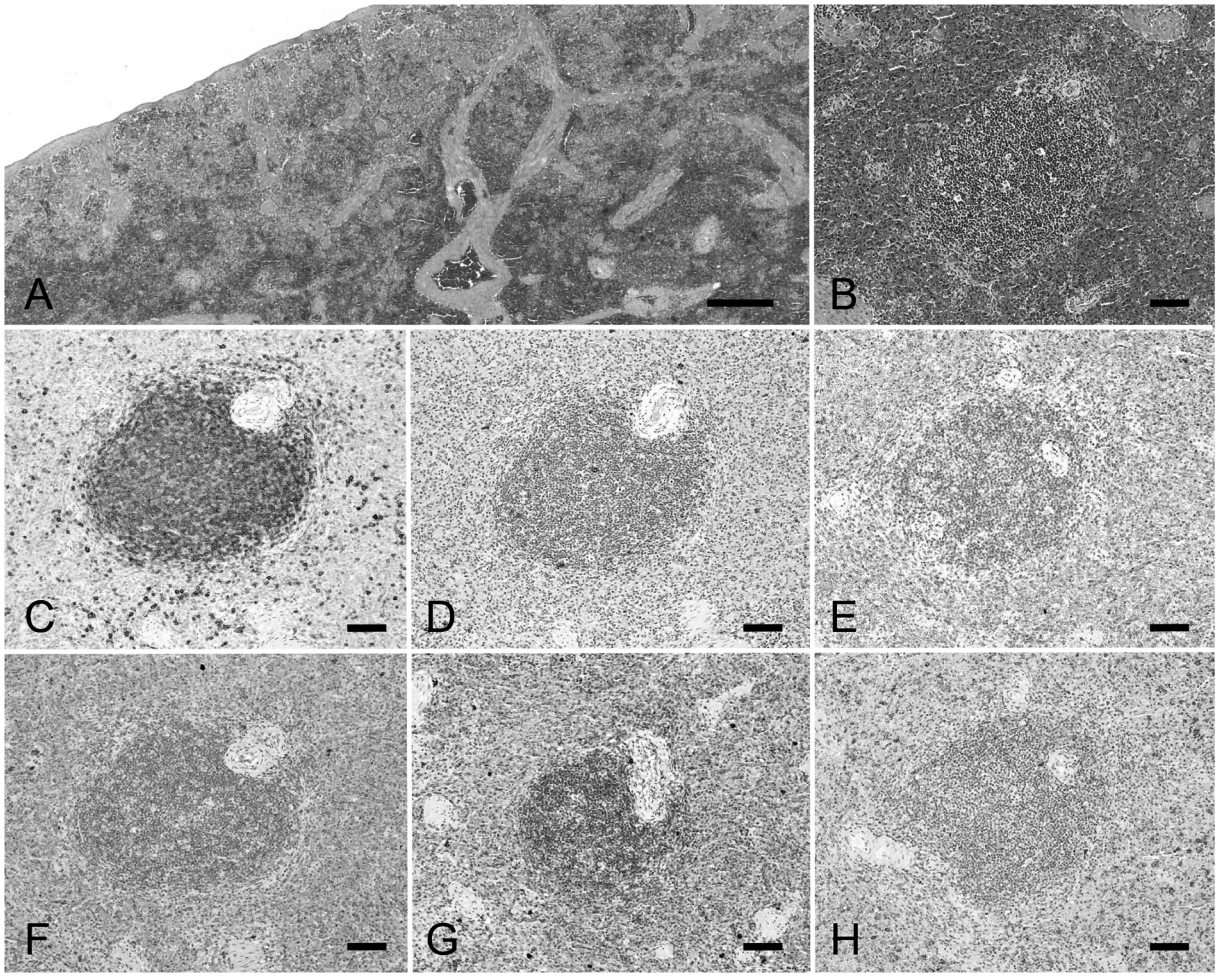

Fig. 2. Hematoxylin and eosin and Immunohistochemical staining of the spleen. Bar=500 mm (A), Bar=100 mm (B–H). Clusters of lymphocytes, or follicular-like structure, were seen, but no mature germinal center was observed (A (lower magnification) and B (higher magnification)). CD3- positive lymphocytes were abundant (C), but the number of B-cell lineage (CD20 (D), PAX5 (E), IgG (F) and IgM (G) –positive cells) was markedly decreased. Anti-Iba-1 (H) immunostaining showed lack of dendritic cells that should compose germinal center, suggesting that follicular structure did not form in the spleen.

Table 1. Primary antibodies used in the present study

Antibody against	Host	Dilution	Antigen retrieval	Source
CD20	rabbit	1: 400	no	Thermo Scientific, CA, U.S.A.
CD3	rabbit	1: 100	autoclave	Dako, Tokyo, Japan
IgG	goat	1: 1,000	autoclave	Bethyl Laboratories, TX, U.S.A.
PAX5	rabbit	ready to use	autoclave	Dako, Tokyo, Japan
Iba-1	rabbit	1: 250	autoclave	Wako, Osaka, Japan

Charles Spaniels [8, 19] and several other breeds [2, 6]. We describe the clinical, histological and immunohistochemical aspects of the case with the confirmation of *P. carinii* by molecular biological analysis. The prominent findings included the marked depletion of B cells in the organs examined and hypoglobulinemia, especially the low concentration of IgG in the serum despite the severe infection.

P. carinii infection, low serum concentrations of IgG and IgA and a decreased number of B cells in the lymphoid tissues of the present case are similar to one report in a miniature dachshund [11]. One difference observed in our case is the later onset of the disease compared to the miniature dachshunds, all of which were under 1 year old when diagnosed. The present case also resembles the case series reported in Cavalier King Charles Spaniels [19], which documented low IgG concentrations and *P. carinii* infection with demodicosis

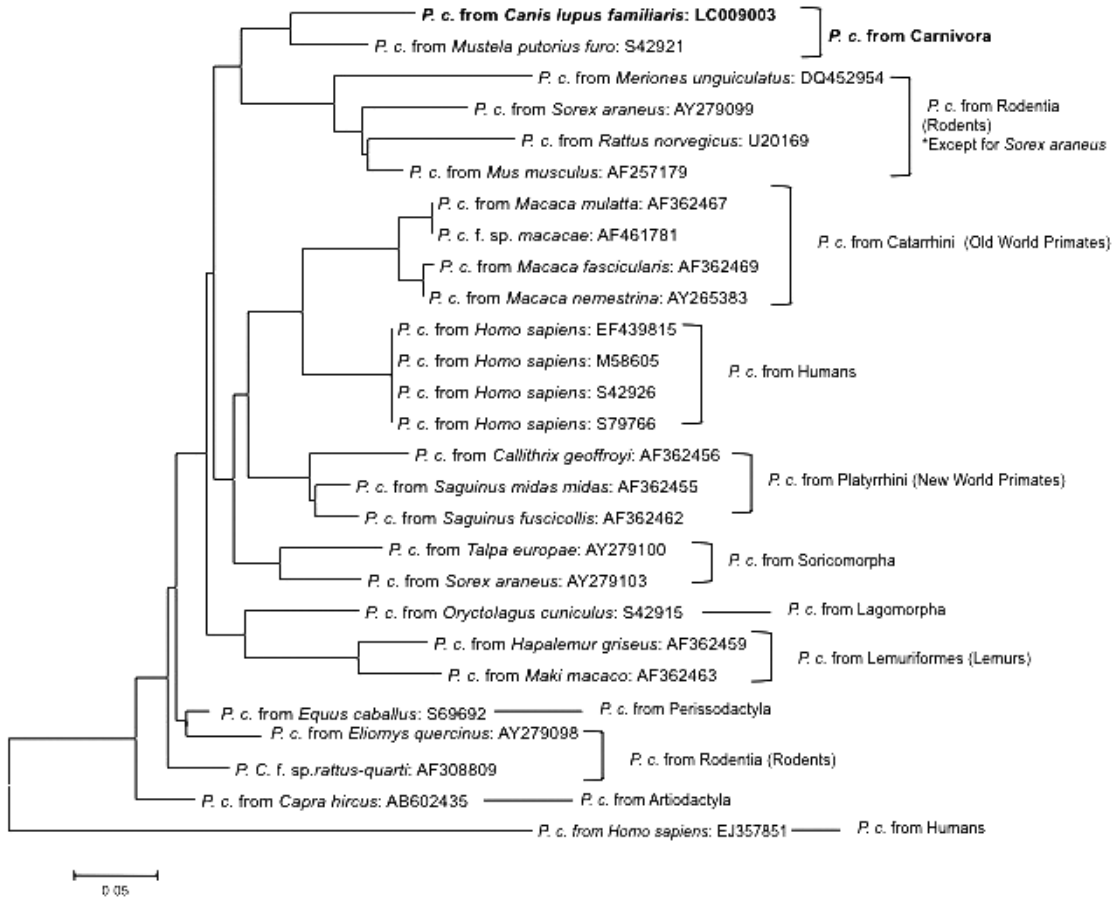

Fig. 3. Phylogenic tree constructed with mtLSU rRNA sequences of *P. carinii* from dog and 26 representative human and animal species with DDBJ accession numbers. The number of the scale bar shows the percentage occurrence in 1,000 bootstrap replicates. The isolate reported in this study is indicated in bold. *P. c.*=*P. carinii*

in several cases. Similar to the present case, most of these dogs were adults.

We were not able to diagnose *P. carinii* infection prior to the patient's death, although it was suspected and treatment was initiated. Although tracheal or bronchial fluids should be collected to facilitate the diagnosis [17], it was impossible to perform the recommended diagnostic procedures in our case due to the patient's severe respiratory distress. The dog responded poorly to treatment with sulfadiazine/trimethoprim, an antibiotic which is reportedly effective against *P. carinii* infection, possibly due to the severity of the pulmonary lesions [19].

Common variable immunodeficiency (CVID), which is characterized by recurrent infection due to a deficiency in antibody production, is the most common primary immunodeficiency in humans [14, 20]. A variety of molecular mechanisms have been identified in humans, indicating that CVID is a group of heterogeneous diseases and is diagnosed by excluding other identifiable primary immunodeficiencies. Some features of the present case resembled human CVID, such as the adult onset, the immunoglobulin deficiency and the opportunistic infection; however, because the serum IgM concentration of this patient was within the reference range and we did not perform a detailed functional assay, whether this case is truly comparable to human CVID is unknown.

On the basis of the immunohistochemical findings, deficient antibody production was attributed to B-cell hypoplasia or depletion of peripheral immune system. Because lymphocytes identified as B cells were observed in the bone marrow, this dog was capable of producing B cells, but it is postulated that peripheral B-cell maturation or expansion was disturbed by an unknown genetic cause in the present case. The finding of B cell absence in the peripheral immune tissue is similar to the pathological finding in horses with CVID, although B cell was not detected in the bone marrow in horses [5]. This difference can be attributed to the wide spectrum of CVID.

The two pathogens identified in this dog, namely *P. carinii* and *Demodex* species, are commonly found in dogs with an underlying immunodeficiency. In dogs, *P. carinii* infection is usually associated with antibody deficiencies [8, 11, 19], although cellular immunodeficiency has also been reported

in a dog with *P. carinii* infection [6]. Based on these previous descriptions in dogs, the findings in our case and the reports from other species [16], *P. carinii* infection can be seen in dogs with either a cellular or humoral immune deficiency. B cells and plasma cells were rarely seen surrounding the *Demodex*-infected hair follicles in this patient. However, these cells were reported to be abundant in the lesions associated with demodicosis in dogs [3, 7], and the absence of antibody-producing cells seemed responsible for the recurring demodicosis in the present case.

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REFERENCES

1. Binanti, D., Mostegl, M. M., Weissenbacher-Lang, C., Nedorost, N. and Weissenböck, H. 2014. Detection of Pneumocystis infections by in situ hybridization in lung samples of Austrian pigs with interstitial pneumonia. *Med. Mycol.* **52**: 196–201. [Medline]
2. Cabañes, F. J., Roura, X., Majó, N., Bragulat, M. R. and Domingo, M. 2000. Pneumocystis carinii pneumonia in a Yorkshire terrier dog. *Med. Mycol.* **38**: 451–453. [Medline] [CrossRef]
3. Day, M. J. 1997. An immunohistochemical study of the lesions of demodicosis in the dog. *J. Comp. Pathol.* **116**: 203–216. [Medline] [CrossRef]
4. Ewing, P. J., Cowell, R. L., Tyler, R. D., MacAllister, C. G. and Meinkoth, J. H. 1994. Pneumocystis carinii pneumonia in foals. *J. Am. Vet. Med. Assoc.* **204**: 929–933. [Medline]
5. Flaminio, M. J., Tallmadge, R. L., Salles-Gomes, C. O. and Matychak, M. B. 2009. Common variable immunodeficiency in horses is characterized by B cell depletion in primary and secondary lymphoid tissues. *J. Clin. Immunol.* **29**: 107–116. [Medline] [CrossRef]
6. Furuta, T., Nogami, S., Kojima, S., Fujita, M., Kamata, H., Kuwabara, M., Ohba, S. and Yoshida, H. 1994. Spontaneous Pneumocystis carinii infection in the dog with naturally acquired generalised demodicosis. *Vet. Rec.* **134**: 423–424. [Medline] [CrossRef]
7. Gortel, K. 2006. Update on canine demodicosis. *Vet. Clin. North Am. Small Anim. Pract.* **36**: 229–241, ix ix. [Medline] [CrossRef]
8. Hagiwara, Y., Fujiwara, S., Takai, H., Ohno, K., Masuda, K., Furuta, T., Nakayama, H., Doi, K. and Tsujimoto, H. 2001. Pneumocystis carinii pneumonia in a Cavalier King Charles Spaniel. *J. Vet. Med. Sci.* **63**: 349–351. [Medline] [CrossRef]
9. Kirberger, R. M. and Lobetti, R. G. 1998. Radiographic aspects of Pneumocystis carinii pneumonia in the miniature Dachshund. *Vet. Radiol. Ultrasound* **39**: 313–317. [Medline] [CrossRef]
10. Kondo, H., Hikita, M., Ito, M. and Kadota, K. 2000. Immunohistochemical study of Pneumocystis carinii infection in pigs: evaluation of Pneumocystis pneumonia and a retrospective investigation. *Vet. Rec.* **147**: 544–549. [Medline] [CrossRef]
11. Lobetti, R. 2000. Common variable immunodeficiency in miniature dachshunds affected with Pneumonocystis carinii pneumonia. *J. Vet. Diagn. Invest.* **12**: 39–45. [Medline] [CrossRef]
12. Ma, L., Imamichi, H., Sukura, A. and Kovacs, J. A. 2001. Genetic divergence of the dihydrofolate reductase and dihydropteroate synthase genes in Pneumocystis carinii from 7 different host species. *J. Infect. Dis.* **184**: 1358–1362. [Medline] [CrossRef]
13. Sakakibara, M., Shimizu, C., Kadota, K. and Hatama, S. 2013. Pneumocystis carinii Infection in a domestic goat (Capra hircus domesticus) with multibacillary paratuberculosis. *J. Vet. Med. Sci.* **75**: 671–674. [Medline] [CrossRef]
14. Salzer, U., Unger, S. and Warnatz, K. 2012. Common variable immunodeficiency (CVID): exploring the multiple dimensions of a heterogeneous disease. *Ann. N. Y. Acad. Sci.* **1250**: 41–49. [Medline] [CrossRef]
15. Settnes, O. P. and Henriksen, S. A. 1989. Pneumocystis carinii in large domestic animals in Denmark. A preliminary report. *Acta Vet. Scand.* **30**: 437–440. [Medline]
16. Steele, C., Shellito, J. E. and Kolls, J. K. 2005. Immunity against the opportunistic fungal pathogen Pneumocystis. *Med. Mycol.* **43**: 1–19. [Medline] [CrossRef]
17. Sukura, A., Saari, S., Järvinen, A. K., Olsson, M., Kärkkäinen, M. and Ilvesniemi, T. 1996. Pneumocystis carinii pneumonia in dogs—a diagnostic challenge. *J. Vet. Diagn. Invest.* **8**: 124–130. [Medline] [CrossRef]
18. Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol. Biol. Evol.* **30**: 2725–2729. [Medline] [CrossRef]
19. Watson, P. J., Wotton, P., Eastwood, J., Swift, S. T., Jones, B. and Day, M. J. 2006. Immunoglobulin deficiency in Cavalier King Charles Spaniels with Pneumocystis pneumonia. *J. Vet. Intern. Med.* **20**: 523–527. [Medline] [CrossRef]
20. Wehr, C., Kivioja, T., Schmitt, C., Ferry, B., Witte, T., Eren, E., Vlkova, M., Hernandez, M., Detkova, D., Bos, P. R., Poerksen, G., von Bernuth, H., Baumann, U., Goldacker, S., Gutenberger, S., Schlesier, M., Bergeron-van der Cruyssen, F., Le Garff, M., Debré, P., Jacobs, R., Jones, J., Bateman, E., Litzman, J., van Hagen, P. M., Plebani, A., Schmidt, R. E., Thon, V., Quinti, I., Espanol, T., Webster, A. D., Chapel, H., Vihinen, M., Oksenhendler, E., Peter, H. H. and Warnatz, K. 2008. The EUROclass trial: defining subgroups in common variable immunodeficiency. *Blood* **111**: 77–85. [Medline] [CrossRef]
21. Weisbroth, S. H. 2006. Pneumocystis: newer knowledge about the biology of this group of organisms in laboratory rats and mice. *Lab. Anim. (NY)* **35**: 55–61. [Medline] [CrossRef]
22. Willmann, M., Müllauer, L., Guija de Arespacochaga, A., Reifinger, M., Mosberger, I. and Thalhammer, J. G. 2009. Pax5 immunostaining in paraffin-embedded sections of canine non-Hodgkin lymphoma: a novel canine pan pre-B- and B-cell marker. *Vet. Immunol. Immunopathol.* **128**: 359–365. [Medline] [CrossRef]