



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

SHORT PAPER

Orchitis in a Cat Associated with Coronavirus Infection

Ó. G. Sigurðardóttir, Ø. Kolbjørnsen and H. Lutz*

Section of Pathology, National Veterinary Institute, P.O. Box 8156, Dep., 0033 Oslo, Norway and

**Clinical Laboratory, Department of Internal Veterinary Medicine, Winterhurestr. 260, 8057 Zurich, Switzerland*

Summary

A case of severe, pyogranulomatous and necrotizing orchitis in a cat, which later succumbed to systemic feline infectious peritonitis (FIP), is described. The 3·5-year-old cat, positive for feline immunodeficiency virus infection, presented with a left testicular enlargement. A few months after castration the animal was humanely destroyed due to declining health. Post-mortem examination revealed inflammatory lesions in abdominal organs and in the brain compatible with FIP. Infection was confirmed with a reverse transcriptase–polymerase chain reaction test and by immunohistochemical demonstration of coronavirus antigen in the affected tissues, including the left testicle. FIP is usually a systemic disease. However, lesions and presenting clinical signs in a single organ system such as the brain are not uncommon. The results of this case study indicate that orchitis, although rare, should be on the list of lesions of FIP.

© 2001 Harcourt Publishers Ltd

Feline infectious peritonitis (FIP) is an immune-mediated disease of young cats, initiated by a viral infection. The causative virus, feline infectious peritonitis virus (FIPV), is a mutant of feline enteric coronavirus (FECV). Whereas FECV has affinity for enterocytes, FIPV replicates in macrophages and spreads throughout the body in infected monocytes. The course of an infection depends in part upon the type and strength of the immune response (Pedersen, 1995; Foley *et al.*, 1998; Kipar *et al.*, 1998; Paltrinieri *et al.*, 1998). Two major clinicopathological forms of FIP are recognized. An effusive form, which occurs in the absence of cell-mediated immunity (CMI), is characterized by the accumulation of fluid in body cavities. A non-effusive form, which develops in the presence of weak CMI, is characterized by proliferative inflammatory lesions in parenchymatous organs, the central nervous system, and the eyes (Pedersen, 1995; Foley *et al.*, 1998; Paltrinieri *et al.*, 1998), the clinical manifestations depending upon the organs affected. The histopathological lesions, which are

similar in both forms of FIP, are characterized by vasculitis and perivasculitis in serous membranes and in various organs. The inflammatory reaction, which is heterogeneous, includes disseminated granulomatous, pyogranulomatous, fibrinonecrotic and lympho-plasmacytic inflammation (Pedersen, 1995; Foley *et al.*, 1998; Kipar *et al.*, 1998).

A 3·5-year-old male, domestic short-hair cat was presented for examination as the result of decreased appetite, cough and malaise. Clinical examination revealed pyrexia, conjunctivitis, and scrotal swelling caused by an enlarged left testicle. The animal gave a positive test reaction for feline immunodeficiency virus (FIV). Antibiotic treatment led to a temporary improvement of general health but no decrease in testicular size. Further examination 2·5 months later revealed a left testicle that was twice the normal size, firm and tender. The right testicle was small and soft. A tumour was suspected in the left testicle and the cat was castrated. Both testicles were fixed in formalin and submitted to the Section of Pathology, National

Veterinary Institute, Oslo. The histopathological diagnosis was a unilateral pyogranulomatous and necrotizing orchitis. The cat received further antibiotic treatment, and again some slight but temporary clinical improvement was seen. However, the animal remained lethargic and when antibiotic therapy was discontinued it gradually lost appetite and developed neurological signs. The cat was humanely destroyed 4½ months after castration.

Post-mortem examination was performed at the Section of Pathology, National Veterinary Institute, Oslo. Macroscopic lesions, which were confined to the abdominal organs, were indicative of non-effusive FIP. The renal capsules were thickened and both kidneys were enlarged due to multiple, often coalescing, white lesions within the renal cortices. The spleen was moderately enlarged and cut surfaces showed prominent lymphoid follicles. The abdominal lymph nodes, especially the mesenteric lymph nodes, were enlarged. The carcass showed signs of severe constipation. Tissue samples from the brain, lung, heart, kidneys, renal capsules, mesenteric lymph nodes, liver and spleen were fixed in 10% neutral buffered formalin, processed by routine methods, sectioned at 4 µm and stained with haematoxylin and eosin (HE). The formalin-fixed testicles submitted previously had been subjected to similar procedures; in addition, however, sections of both testicles were stained by the periodic acid-Schiff (PAS), Gram and Ziehl-Neelsen (ZN) methods for fungi, for gram-positive and gram-negative bacteria, and for acid-fast bacteria, respectively.

Histological examination revealed a severe subacute to chronic inflammation in the left testicle (Fig. 1). There was effacement of the normal architecture due to a multifocal to coalescing inflammatory cell infiltration. Many large, necrotic and purulent foci were surrounded by macrophages, lymphocytes and plasma cells. There were also several lymphoid follicles with germinal centres throughout the tissue. Scattered intact but degenerated seminiferous tubules were present. The inflammation involved the mediastinum testis and, multifocally, the testicular capsule. In the epididymis, a mild, mixed inflammatory cell reaction was present, especially around and sometimes involving the walls of veins. A few mononuclear inflammatory cells were seen around one vessel in the mediastinum of the right testicle, and the seminiferous tubules were atrophic. No fungi or bacteria were detected histologically within the lesions of the left testicle. Histopathological lesions compatible with FIP were present in the visceral peritoneum, kidneys, renal capsules, mesenteric

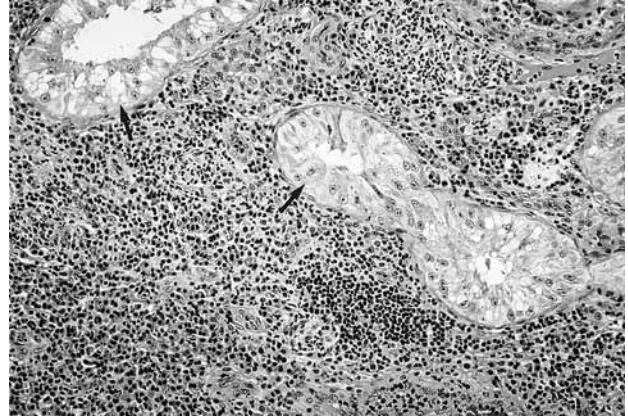


Fig. 1. Orchitis in a cat. A severe purulent and lymphoplasmacytic inflammation in the interstitial tissue of the left testicle. Seminiferous tubules are degenerated (arrows). HE. $\times 114$.

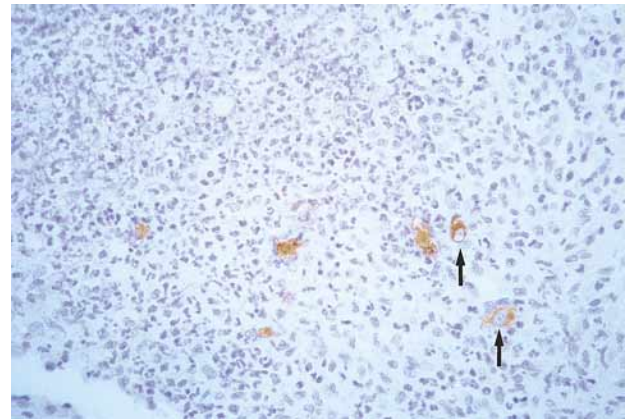


Fig. 2. Positive labelling for coronavirus in a focus of pyogranulomatous inflammation in the left testicle. In places, the positive labelling is clearly demonstrated within the cytoplasm of macrophages (arrows). ABC. $\times 114$.

lymph nodes, brain and lungs. In addition, occasional protozoal cysts were seen in the brain, giving a positive immunohistochemical reaction with *Toxoplasma gondii* antiserum. A very mild inflammatory cell reaction was seen around one of these cysts, but the remainder were inert.

The avidin-biotin-peroxidase complex (ABC) method for demonstrating coronavirus antigen was performed on formalin-fixed, paraffin wax-embedded tissue. A monoclonal antibody, 63I, produced by immunizing mice with transmissible gastroenteritis virus (TGEV) capsid protein p56, was used (Tammer *et al.*, 1995). With this method, coronavirus antigen was demonstrated in the left testicle (Fig. 2), choroid plexus of the brain, both kidneys, renal capsules, spleen and a mesenteric

lymph node. Coronavirus antigen was not demonstrated in the right testicle or liver.

A one-tube reverse transcriptase–polymerase chain reaction test for feline coronavirus, based on the amplification of a sequence of the 7 b gene common to the known feline coronavirus strains (Gut *et al.*, 1999), was run on formalin-fixed, paraffin wax-embedded tissue from the brain, kidneys, renal capsules and left testicle. All the tissues were positive except brain.

The testicle is an immunologically privileged organ, in which local factors prevent autoimmunity to spermatozoal antigens and protect the tissue from deleterious systemic effects. These protective factors include the blood–testis barrier and immunological regulation. The blood–testis barrier has an epithelial and an endothelial component. The epithelial barrier of Sertoli cells and smooth muscle cells prevents contact between spermatogenic cells and blood constituents (Holash *et al.*, 1993; Mukasa *et al.*, 1995). The endothelial barrier is provided by the testicular capillaries, presumably in conjunction with the Leydig cells. Although not as efficient a barrier as the capillaries of the brain, the testicular capillaries are less permeable than most vascular beds (Holash *et al.*, 1993). In addition to the blood–testis barrier, local immune mechanisms play a role in the “down-regulation” of autoimmunity and inflammatory responses (Mukasa *et al.*, 1995). Disruption of the blood–testis barrier, due for example to local trauma or inflammation, may lead to the breakdown of spermatozoal sequestration. Spermatogenic antigens then become exposed to the immune system, leading to the formation of anti-sperm antibodies. An immune-mediated orchitis, characterized by lympho-plasmacytic infiltration, tubular destruction and foreign body granulomatous reaction to dislodge sperm, may then compound the initial testicular lesion (Ladds, 1993; Mukasa *et al.*, 1995).

Orchitis is relatively rare in animals, cats being no exception. Bacteria, reaching the testicles via the blood, ductus deferens or wounds of the scrotal skin, are the most common causes of inflammation, together with trauma (Ladds, 1993; Foster *et al.*, 1996). Periorchitis is a recognized clinical manifestation of the effusive form of FIP. The lesion in the tunica vaginalis is an extension along the inguinal canal of the inflammatory reaction in the peritoneal cavity (Ladds, 1993; Pedersen, 1995). A bilateral orchitis in a cat, ascribed to FIP, was recently reported by Foster *et al.* (1996), but coronavirus infection was not confirmed; the major clinical sign in this cat was testicular enlargement,

but gradually other organ systems became affected.

The histomorphological lesions in the testicle of the cat in the present study, together with the demonstration of coronavirus, strongly support the diagnosis of a FIPV-induced orchitis. Two types of hypersensitivity immune responses appear to play a role in the development of FIP lesions, namely types III and IV (Paltrinieri *et al.*, 1998). In type-III hypersensitivity, pathogenic immune-complexes (ICs) are formed and deposited in and around blood vessels. This leads to a cascade of events culminating in vasculitis, disseminated intravascular coagulation, tissue inflammation and necrosis of the Arthus type (Foley *et al.*, 1998; Kipar *et al.*, 1998; Paltrinieri *et al.*, 1998). Since orchitis is not a common feature of FIP, predisposing factors are likely to have played a role in the present case. Inflammation, due for example to trauma, may have disrupted the endothelial blood–testis barrier, promoting the deposition of circulating ICs or ICs production *in situ*. Disruption of the epithelial blood–testis barrier would further exacerbate the inflammatory cell reaction.

Concurrent infections, especially with feline leukaemia virus (FeLV), are potential predisposing factors for the development of FIP in coronavirus-infected cats (Pedersen, 1995; Kipar *et al.*, 1998). Infection with the FIV may, in the same way as FeLV, adversely affect CMI, thereby predisposing to FIP lesions (Kipar *et al.*, 1998). The cat in the present study was positive for FIV but, other than FIP, had no lesions indicative of an immunosuppressed state. FIV infection was reported by Davidson *et al.* (1993) to predispose to generalized infection with *Toxoplasma gondii*. However, the *Toxoplasma gondii* cysts in the brain of the cat in the present case were presumably of no clinicopathological consequence.

Although lesions of non-effusive FIP may occur in a single organ system such as the brain, eye or the intestine, a systemic disease usually develops gradually (Foley *et al.*, 1998). We propose that the testicles be included on the list of single organ systems affected in FIP. The differential diagnosis of orchitis in cats should include FIP, which would necessitate a guarded prognosis.

References

- Davidson, M. G., Rottman, J. B., English, R. V., Lappin, M. R. and Tompkins, M. B. (1993). Feline immunodeficiency virus predisposes cats to acute generalized toxoplasmosis. *American Journal of Pathology*, **143**, 1486–1497.
- Foley, J. E., Lapointe, J. M., Koblik, P., Poland, A. and Pedersen, N. C. (1998). Diagnostic features of clinical

- neurologic feline infectious peritonitis. *Journal of Veterinary Internal Medicine*, **12**, 415–423.
- Foster, R. A., Caswell, J. L. and Rinkardt, N. (1996). Chronic fibrinous and necrotic orchitis in a cat. *Canadian Veterinary Journal*, **37**, 681–682.
- Gut, M., Leutenegger, C., Huder, J., Pedersen, N. and Lutz, H. (1999). One-tube fluorogenic reverse transcription-polymerase chain reaction for the quantitation of feline coronavirus. *Journal of Virological Methods*, **77**, 37–46.
- Holash, J. A., Harik, S. I., Perry, G. and Stewart, P. A. (1993). Barrier properties of testis microvessels. *Proceedings of the National Academy of Sciences of the United States of America*, **90**, 11069–11073.
- Kipar, A., Bellmann, S., Kremendahl, J., Köhler, K. and Reinacher, M. (1998). Cellular composition, coronavirus antigen expression and production of specific antibodies in lesions in feline infectious peritonitis. *Veterinary Immunology and Immunopathology*, **65**, 243–257.
- Ladds, P. W. (1993). The male genital system. In: *Pathology of Domestic Animals*, 4th Edit., K. V. F. Jubb, P. C. Kennedy and N. Palmer, Eds, Academic Press, New York, 477–478, 500–502.
- Mukasa, A., Hiromatsu, K., Matsuzaki, G., O'Brien, R., Born, W. and Nomoto, K. (1995). Bacterial infection of the testis leading to autoaggressive immunity triggers apparently opposed responses of $\alpha\beta$ and $\gamma\delta$ T cells. *Journal of Immunology*, **155**, 2047–2056.
- Paltrinieri, S., Cammarata, M. P., Cammarata, G. and Comazzi, S. (1998). Some aspects of humoral and cellular immunity in naturally occurring feline infectious peritonitis. *Veterinary Immunology and Immunopathology*, **65**, 205–220.
- Pedersen, N. C. (1995). An overview of feline enteric coronavirus and infectious peritonitis virus infections. *Feline Practice*, **23**, 7–20.
- Tammer, R., Evensen, O., Lutz, H. and Reinacher, M. (1995). Immunohistological demonstration of feline infectious peritonitis virus antigen in paraffin-embedded tissues using feline ascites or murine monoclonal antibodies. *Veterinary Immunology and Immunopathology*, **49**, 177–182.

[Received, June 22nd, 2000]
[Accepted, September 6th, 2000]