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SHORT PAPER

Systemic Toxoplasmosis and Concurrent Porcine Circovirus-2 Infection in a Pig

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Summary

Systemic toxoplasmosis and concurrent infection with porcine circovirus-2 (PCV-2) was diagnosed in a fattening pig. Clinical examination of the herd showed that up to 30% of the pigs of this weight group suffered from severe respiratory signs including sneezing and coughing, with a mortality rate of up to 5%. Gross necropsy examination revealed severe interstitial pneumonia and generalized lymphadenopathy. On microscopical examination there was necrotizing inflammation of the lung, adrenal glands and lymph nodes, associated with lymphoid depletion, cytoplasmic basophilic botryoid inclusion bodies and protozoal microorganisms. Infection with *Toxoplasma gondii* was confirmed by immunohistochemistry (IHC). Polymerase chain reaction analysis, insitu hybridization and IHC confirmed systemic PCV-2 infection. These findings, associated with the respiratory signs and lesions in lymphoid tissues, are characteristic for post-weaning multisystemic wasting syndrome (PMWS). In this case, immunosuppression by PCV-2 may have triggered systemic toxoplasmosis, or immune stimulation caused by coinfection with *T. gondii* may have caused extensive replication of PCV-2.

Keywords: concurrent infection; pig; porcine circovirus-2; *Toxoplasma gondii*

During recent decades, porcine circovirus-2 (PCV-2) infection has become of major economic importance to the pig industry. Retrospective studies have demonstrated that the presence of PCV-2 can be traced back to 1962 in Germany (Jacobsen *et al.*, 2009) and since then the number of diseases associated with this infection has rapidly expanded (Carman *et al.*, 2006; Opriessnig *et al.*, 2007). In addition to post-weaning multisystemic wasting syndrome (PMWS) and porcine dermatitis and nephritis syndrome (PDNS), PCV-2 infection has been associated with proliferative and necrotizing pneumonia (Chae, 2005), cerebellar vasculitis (Seeliger *et al.*, 2007), granulomatous enteritis (Chae, 2005), reproductive failure with abortion and premature farrowing (West *et al.*, 1999; Allan and Ellis, 2000; Park *et al.*, 2005) and neonatal losses with tremor (Stevenson *et al.*, 2001) or myocarditis (West *et al.*, 1999; Mikami *et al.*, 2005). Pigs infected with PCV-2 often develop concurrent infections with agents such as porcine parvovirus (PPV), swine influenza virus (SIV), *Mycoplasma hyopneumoniae* (Morin *et al.*, 1990; Ellis *et al.*, 1999; Larochelle *et al.*, 1999; Harms *et al.*, 2001), porcine respiratory and reproductive syndrome virus (PRRSV; Harms *et al.*, 2001), Aujezsky's disease virus (Rodriquez-Arrioja *et al.*, 1999), *Chlamydophila* (Carrasco *et al.*, 2000) or fungi such as *Pneumocystis carinii* (Clark, 1997).

Pallarés *et al.* (2002) described bacterial septicaemia and pneumonia in cases of PMWS. Bacterial septicaemia was triggered by *Streptococcus suis*, *Salmonella* spp., *Arcanobacterium pyogenes*, *Haemophilus parasuis*, *Actinobacillus suis*, *Escherichia coli* or *Erysipelothrix rhusiopathiae* and concurrent bacterial pneumonia was caused by *Pasteurella multocida*, *Bordetella bronchiseptica* or *Actinobacillus pleuropneumoniae*.

Toxoplasmosis is a zoonotic disease with worldwide distribution (Dubey and Beattie, 1988). It is recognized as the third leading cause of death caused by food-borne diseases in people in the USA (Jones

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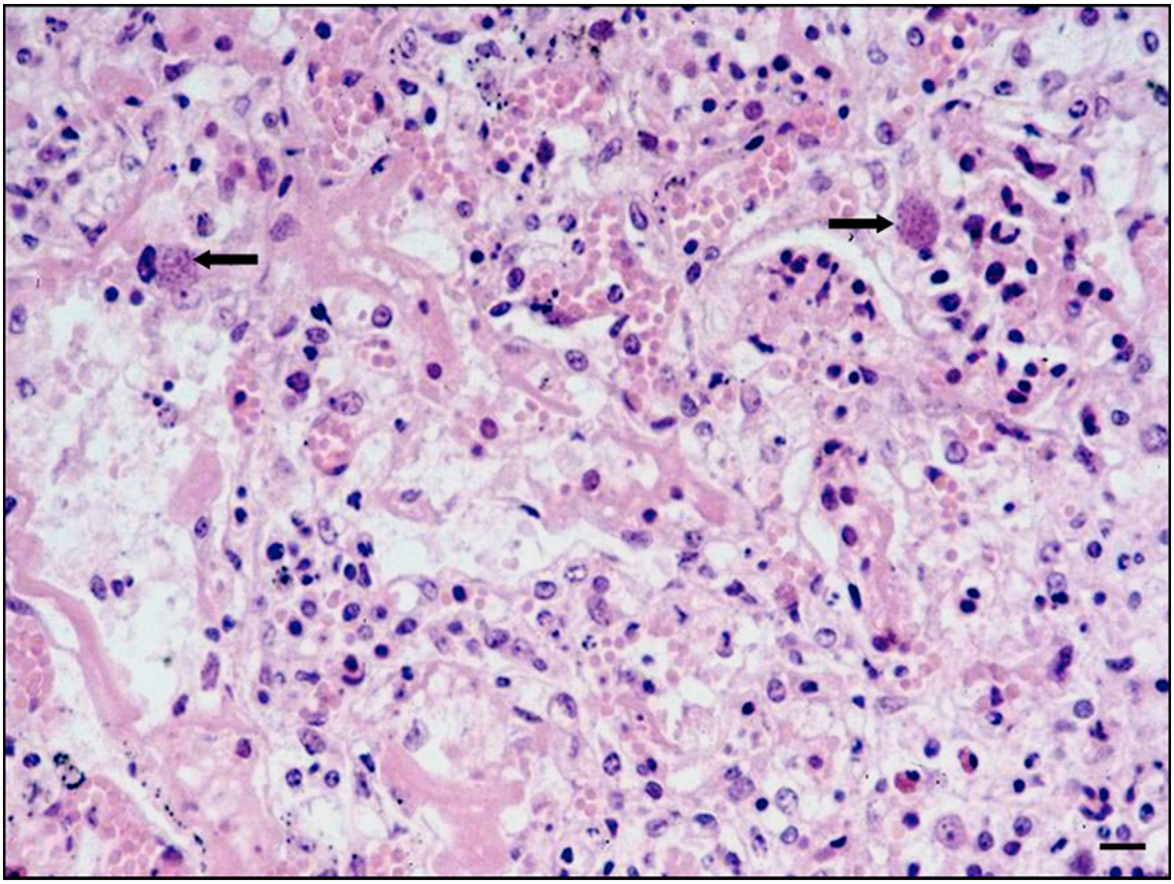

Fig. 1. Interstitial and necrotizing pneumonia with protozoal organisms (arrows). HE. Bar, 40 μm.

et al., 2001). Among meat producing animals, pigs are considered to be the most important source of human infection with *Toxoplasma gondii* in the USA (Dubey, 1986). In most porcine cases, infection with *T. gondii* is subclinical, while systemic toxoplasmosis has been reported only rarely in pigs (Weissenböck and Dubey, 1993). Affected animals show dyspnoea, cyanosis and fever. Morphological lesions comprise interstitial pneumonia, non-suppurative meningoencephalomyelitis, necrotizing hepatitis, adrenalitis and lymphadenitis. In other species, such as dogs with canine distemper virus infection, systemic toxoplasmosis often follows underlying immunosuppressive disease (Ehrensperger and Pospischil, 1989; Beineke *et al*., 2009); however, such primary infection has not been shown in the pig. The present report describes the clinical, morphological and aetiological findings in a pig with a concurrent PCV-2 infection and associated systemic toxoplasmosis.

A 3.5-month-old, castrated male pig weighing approximately 27 kg had a history of severe respiratory signs including sneezing and coughing and died suddenly. Clinical examination of the herd revealed that approximately 30% of the animals of this weight group suffered from similar respiratory signs, with a mortality rate of 5%. All pigs had been vaccinated against *M. hyopneumoniae* and PRRS. The pig examined had not been treated with antibiotics.

The animal was submitted for necropsy examination. The pig was in moderate bodily condition. The lung was collapsed with a mottled light to dark red lobular appearance and a diffusely firm consistency. Atelectatic areas were noted cranioventrally. On cut surface there was mucopurulent exudation from the deeper airways of the cranial lobes. The regional and other visceral lymph nodes were enlarged with a bulging and oedematous cut surface.

Samples of brain, thymus, thyroid glands, oesophagus, nasal mucosa, lung, heart, lymph nodes (pulmonary, mesenteric and iliac), stomach, intestine, pancreas, spleen, liver, kidneys, adrenal glands, skin, skeletal muscles and bone marrow were fixed in 10% neutral buffered formalin, processed routinely, embedded in paraffin wax, sectioned (5 μm) and stained with haematoxylin and eosin (HE).

Microscopical examination revealed severe diffuse interstitial pneumonia with infiltration of macrophages and fewer neutrophils and lymphocytes.

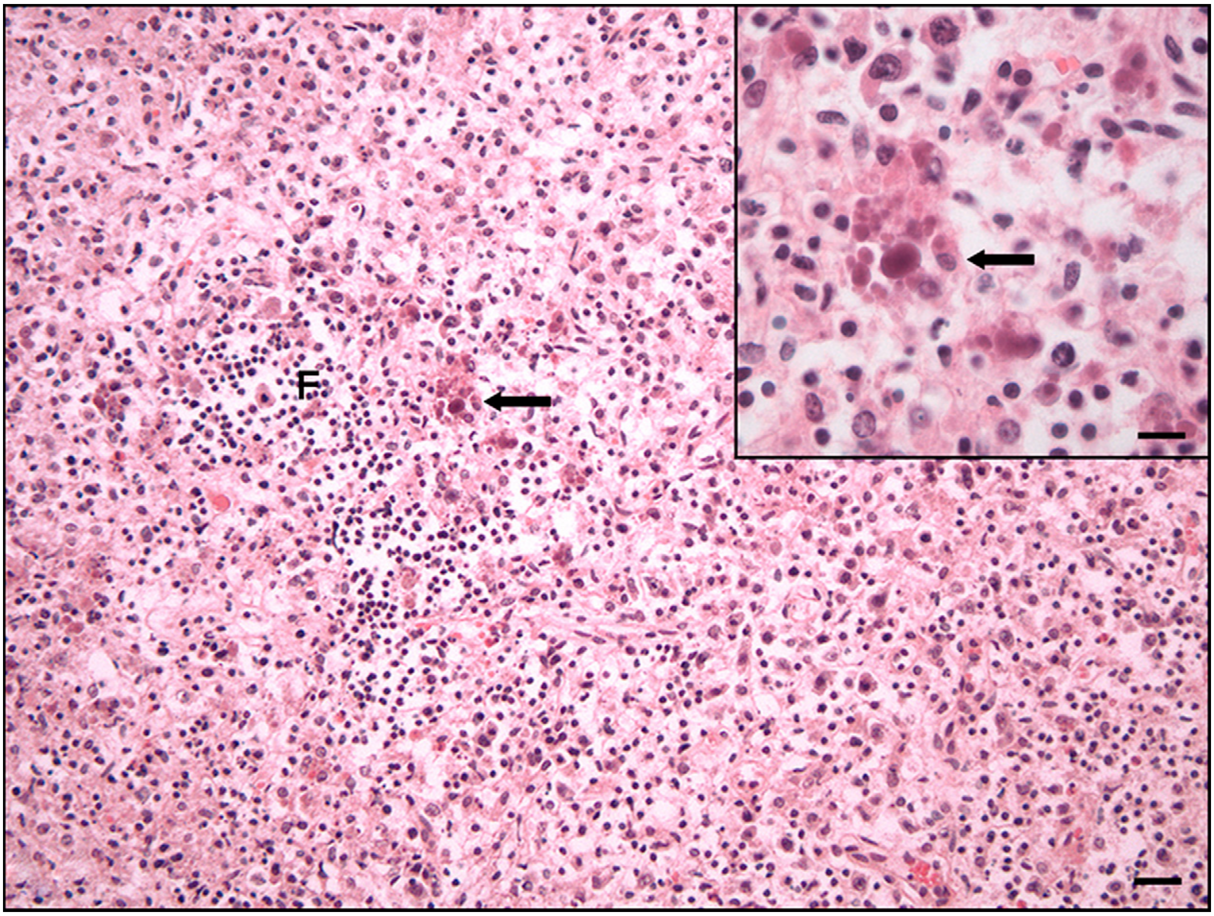

Fig. 2. Pulmonary lymph node with marked lymphoid depletion (F, follicle) and basophilic cytoplasmic botryoid inclusion bodies (arrow). HE. Bar, 70 μm. Inset: follicular histiocytic cell with basophilic cytoplasmic botryoid inclusion bodies. HE. Bar, 15 μm.

There was also severe necrotizing pneumonia with extensive desquamation of type 2 pneumocytes into the alveolar spaces. In addition, severe diffuse alveolar and mild interstitial pulmonary oedema was observed. Within necrotic areas there were intracellular clusters of protozoal microorganisms that were most likely within macrophages (Fig. 1). In addition, there were extracellular tachyzoites characterized by a round to ovoid shape, an eccentric nucleus and a variable diameter (3–6 μm). Lymph nodes had marked lymphoid depletion and multiple foci of fibrinonecrotic inflammation. Numerous macrophages contained cytoplasmic protozoal microorganisms. Additionally, cytoplasmic basophilic botryoid inclusion bodies indicative of PCV-2 infection were observed in follicular histiocytes (Fig. 2). There was moderate lymphoid depletion of the spleen. Multifocal, moderate, necrotizing adrenalitis with cytoplasmic protozoal microorganisms in macrophages and extracellular tachyzoites was observed (Fig. 3a). Kidneys, heart, liver, pancreas, colon and central nervous system displayed mild to moderate perivascular lymphohistiocytic infiltration. Intranuclear basophilic inclusion bodies indicating PCMV infection were not detected in the epithelium of the nasal mucosa and glands.

In-situ hybridization and immunohistochemistry (IHC) were carried out on selected tissue sections. For IHC, a polyclonal rabbit antibody against *T. gondii* (Quartett, Berlin, Germany; Brack *et al.*, 1998) diluted 1 in 80 in phosphate buffered saline (PBS), and a murine monoclonal antibody specific for PCV-2 (Clone 35A9, Ingenasa, S. A. Madrid, Spain), diluted 1 in 400 in PBS were applied using the avidin–biotin–peroxidase complex (ABC) method (Vector Laboratories, Burlingame, USA). In-situ hybridization was performed as described previously (Rosell *et al.*, 1999; Seeliger *et al.*, 2007; Jacobsen *et al.*, 2009) using a PCV-2-specific, digoxigenin-labelled oligonucleotide probe (DIG-5′-CCTTCCTCATTACCCTCC TCGCCAACAATAAAATAATCAAA-3′) that was designed from the sequence of PCV-2 open reading frame 1 (nucleotides 168–208, Genbank accession number AF027217). Lung tissue and pulmonary

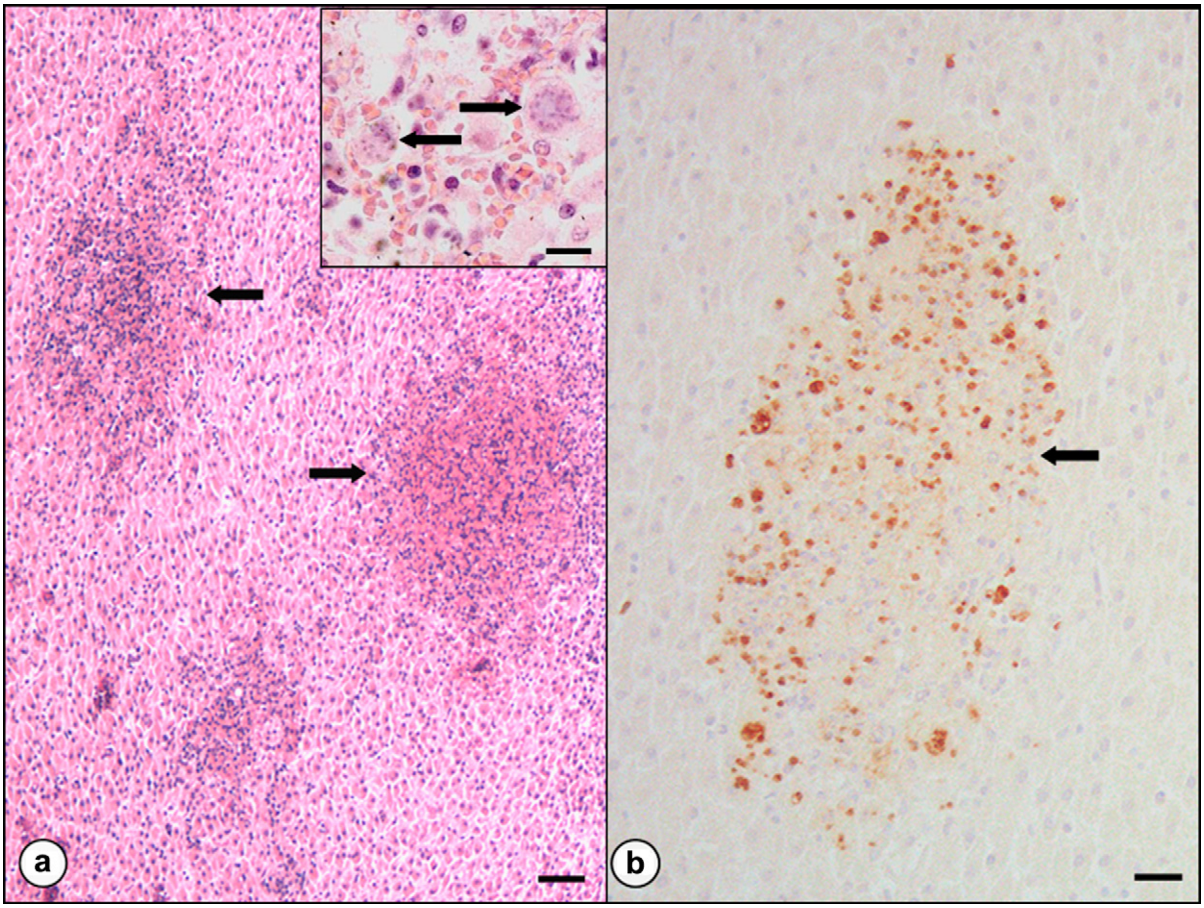

Fig. 3. (a) Adrenal gland with multifocal necrotizing inflammation (arrows). HE. Bar, 70 μm. Inset: protozoal organisms (arrows). HE. Bar, 40 μm. (b) Immunohistochemical demonstration of *T. gondii* antigen in necrotic areas of the adrenal gland. IHC. Bar, 50 μm.

lymph nodes were also processed for polymerase chain reaction (PCR) analysis. A multiplex PCR specific for various respiratory agents including PRRS (EU- and US-subtype), PCV-2, swine influenza virus (SIV), porcine respiratory coronavirus (PRCV), porcine cytomegalovirus (PCMV), *M. hyopneumoniae* and *M. hyorrhinis* was performed (Harder and Hübert, 2004).

Immunohistochemically, *T. gondii* antigen was detected in the cytoplasm of macrophages in adrenal glands (Fig. 3b), intestine, heart, lymph nodes and lung (Fig. 4a). IHC revealed PCV-2 antigen in the cytoplasm of macrophages in inflammatory infiltrates of the kidneys, spleen, pancreas, liver, adrenal glands and lung (Fig. 4b). In addition, PCV-2 antigen was found in follicular histiocytes and macrophages of the lymph nodes, tonsils and gut-associated lymphoid tissue (GALT). PCV-2 genome fragments were detected in similar locations. Multiplex PCR analysis resulted in amplification of genome fragments of PCV-2 and PCMV in lung and pulmonary lymph nodes. No bacteria were isolated from samples of lung.

These investigations supported the diagnosis of systemic toxoplasmosis and concurrent PCV-2 infection in this animal. Respiratory disease, lymphoid depletion and interstitial pneumonia associated with the presence of PCV-2 were compatible with PMWS (Opriessnig *et al.*, 2007). However, the signs of respiratory infection may also have been attributed to systemic toxoplasmosis. Necrotizing pneumonia, necrotizing lymphadenitis and adrenal necrosis were caused by *T. gondii* as demonstrated immunohistochemically, and these changes are similar to those described previously in pigs with toxoplasmosis (Weissenböck and Dubey, 1993). However, the hepatic necrosis that commonly occurs in systemic toxoplasmosis (Weissenböck and Dubey, 1993) was not found in the present case. Protozoal microorganisms were not detected in the skeletal muscles examined.

A major finding in pigs with PCV-2 infection is depletion of lymphoid tissues (Sato *et al.*, 2000; Krakowka *et al.*, 2002) associated with a decrease in $CD4^+$ T lymphocytes and B lymphocytes in the peripheral blood (Segalés *et al.*, 2001). These changes

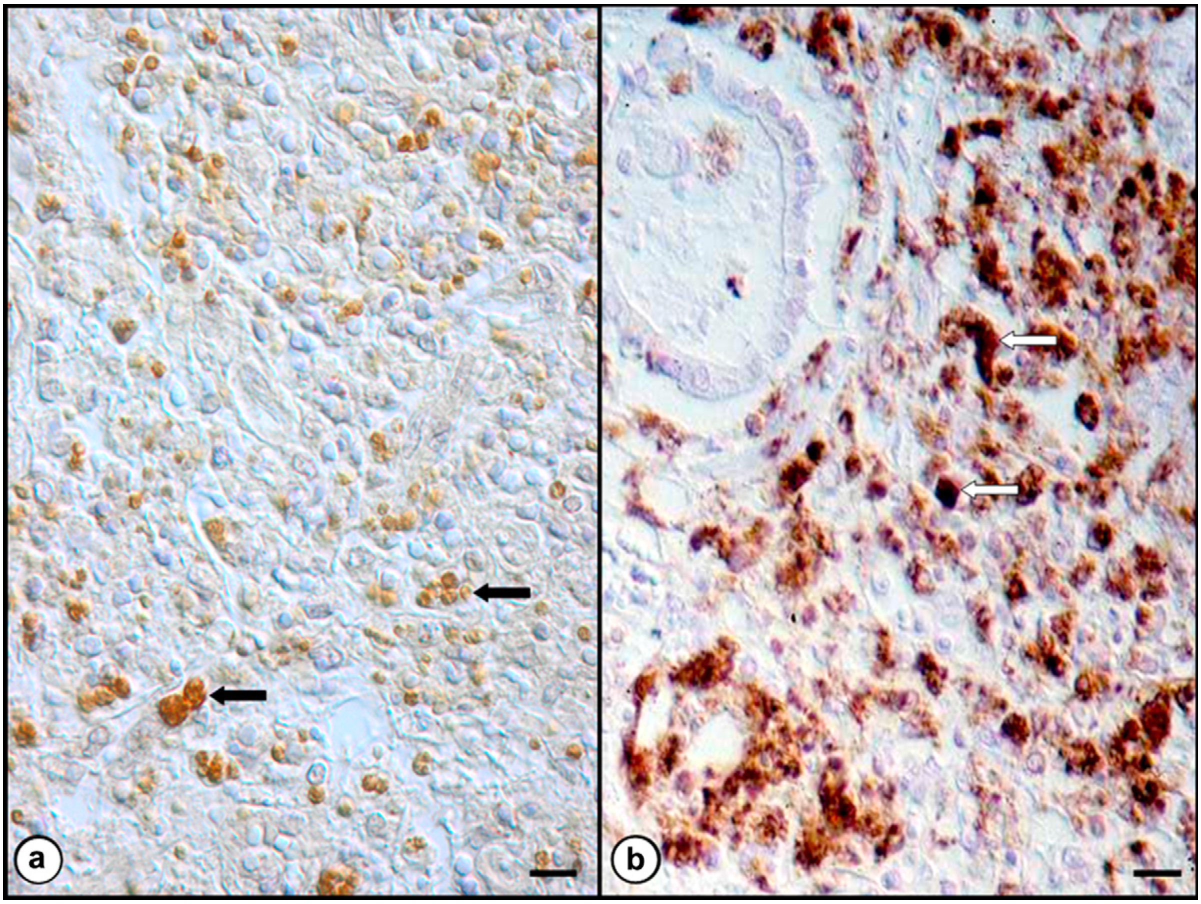

Fig. 4. (a) Immunohistochemical labelling of *T. gondii* antigen in the lung (arrows). IHC. Bar, 20 μm. (b) Immunohistochemical labelling of PCV-2 antigen in numerous inflammatory cells of the lung (arrows). IHC. Bar, 20 μm.

are collective evidence of immunosuppression, which facilitates the development of coinfections (Segalés *et al*., 2004). In the present case, primary PCV-2 infection may therefore have predisposed to the development of secondary systemic toxoplasmosis. Alternatively, stimulation of the immune system by vaccination or concurrent infection, in this case with *T. gondii*, may have resulted in proliferation of target cells allowing extensive replication of PCV-2 (Krakowka *et al*., 2001; Jacobsen *et al*., 2009).

The mode of infection and source of *T. gondii* was not determined. Contamination of food with feline faeces or ingestion of infected rodents was considered the most likely source of infection. Cats are the main reservoir and definitive hosts of *T. gondii* excreting millions of oocysts (Dubey and Frenkel, 1972). After ingestion of oocysts by intermediate hosts, tissue cysts are formed in 5–7 days (Dubey, 1997; Dubey *et al*., 1997). Man and animals become infected mainly by ingesting tissue cysts or oocysts. Consumption of raw or undercooked meat products containing *T. gondii* tissue cysts (bradyzoites) or ingestion of food or water contaminated with infectious oocysts (sporozoites) from cat faeces are risk factors associated with *T. gondii* infection (Hill *et al*., 2006).

In most adult human patients *T. gondii* infection does not cause serious illness. However, devastating disease may occur in immunocompromised individuals such as those with acquired immune deficiency syndrome (AIDS) (Dubey *et al*., 1998). A similar situation occurs in animals with acquired immunodeficiency including dogs with canine distemper virus infection (Ehrensperger and Pospischil, 1989; Beineke *et al*., 2009). *Toxoplasma* encephalitis has emerged as a major cause of morbidity and mortality in patients with AIDS, ranging between 5–10% in the US and 25–50% of AIDS patients in Europe (Suzuki, 1993). In man, congenital infection can lead to abortion and stillbirth in 10% of affected fetuses, or blindness and mental retardation of children (Hill and Dubey, 2002). Due to the fatal outcome in human patients, detection and removal of infected swine carcasses from the food chain is considered an important food safety issue (Hill *et al*., 2006). *T. gondii* infection is highly prevalent among pigs in many countries. With respect to the increased numbers of PCV-2 infections worldwide, systemic toxoplasmosis should be considered as a potential PCV-2 associated disease in pigs.

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