한국가축위생학회지 제41권 제1호 (2018)
Korean J Vet Serv, 2018, 41(1), 51-55
ISSN 1225-6552, eISSN 2287-7630
https://doi.org/10.7853/kjvs.2018.41.1.51

Korean Journal of
Veterinary Service
Available online at http://kjves.org

< Case Report >

Pathological findings of the mixed infection with canine distemper virus and *Streptococcus canis* on farmed badger

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(Received 23 February 2018; revised 14 March 2018; accepted 19 March 2018)

Abstract

Herein, we report a case of badgers showing high morbidity and mortality rate due to the mixed infection of canine distemper virus (CDV) and *Streptococcus canis* (*S. canis*) in a farm where wild animal, badger, is being reared for herbal medicine. During the period of about one month, 120 out of 320 badgers showed severe respiratory symptoms and died, and 3 bodies were submitted to the Animal and Plant Quarantine Agency for disease diagnosis. The lung with the most severe necropsy findings failed to collapse and showed dark reddening and had yellowish nodules on the cut surface. The characteristic and common histopathologic findings include multifocal necrosis with hemorrhage of the lung, severe lymphoid depletion of the spleen and intracytoplasmic or intranuclear inclusion bodies in almost all organs. Finally, CDV and *S. canis* were identified by immunohistochemistry and bacterial isolation, respectively. This is the first mixed infection case of CDV and *S. canis* in badgers being raised on the farm.

Key words : Badger, Canine distemper virus (CDV), Mixed infection, Pathology, *Streptococcus canis* (*S. canis*)

INTRODUCTION

Canine distemper virus (CDV), a relatively large single-stranded RNA virus, is a member of the genus *Mobilivirus* of the family *Paramyxoviridae*. CDV causes canine distemper (CD), a highly contagious systemic disease affecting terrestrial carnivores including Canidae, Mustelidae, Ailuridae, Mephitidae, and Ursidae (Deem et al, 2000). CDV spreads by aerosol droplet secretions from infected animals and enters the respiratory tract (Ford, 2012). Virus initially replicates in the tonsils and lymphatic tissue, which is followed by infection of respiratory, gastro-intestinal tract, urinary tract, and central nervous system (Dungworth, 1993). In the early stage of typical infection, a transient fever usually occurs and there may be accompanied by anorexia, nasal discharge, and lethargy (Ford, 2012). In addition, multisystemic disease with severe neurological signs can occur (Vandevelde et al, 2005; Moretti et al, 2006). Affected animals with CDV are prone to opportunistic infections as a consequence of generalized lymphoid depletion and profound immunosuppression (Rudd et al, 2006; Chvala et al, 2007). In previous cases, double infection with the *Toxoplama gondii* and CDV and triple infection with canine adenovirus (CAV) type 2, *Mycoplasma cynos* and CDV in a dogs were reported (Chvala et al, 2007; Aguiar et al, 2012).

Streptococcus canis (*S. canis*) is generally an opportunist pathogen and a group G beta-hemolytic species of *Streptococcus* (Facklam, 2002). *S. canis* is important to the skin and mucosal health of cats and dogs, but under certain circumstances, the bacteria have been reported to cause diseases in a variety of mammals (Facklam, 2002). The lungs have been considered the organ seriously af-

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fected by *S. canis* infection, and acute infection appeared to be superimposed on chronic preexisting pulmonary infection (Murase et al, 2003).

Mustelids are the most susceptible to CDV disease among the species. The badger is a carnivore belonging to the family Mustelidae (Phillippa et al, 2008). In Korea, CD case has been reported in wild badgers with single infection in 1997 (Kim et al, 1997). This is the first report of the mixed infection with CDV and *S. canis* in reared badgers through pathological findings and etiological examination.

CASE

Three hundred twenty badgers raised for herbal medicine were kept on a farm located in Gyeongbuk province, republic of Korea. All of them did not have any vaccination history. About one hundred twenty badgers showed by dyspnea, reduced appetite, weight loss, half-closed eyes with lacrimation, and death over a month period. Three 5-year-old female badgers among them were submitted to the Animal and Plant and Quarantine Agency for laboratory diagnosis.

At necropsy, the lungs failed to collapse and were rubbery and mottled dark red appearances on the surface. On the cut surface of the lungs, yellowish nodules were multifocally observed (Fig. 1A). The tissue samples including brain, lung, spleen, liver, kidney, stomach, and urinary bladder were fixed in 10% neutral buffered formalin, processed routinely, and embedded in paraffin wax. Tissue sections (4 μm) were cut and stained with hematoxylin and eosin. Immnunohistochemistry (IHC) was performed using mouse monoclonal antibody against canine distemper virus (MCA1893, AbD Serotec, Duesseldorf, Germany).

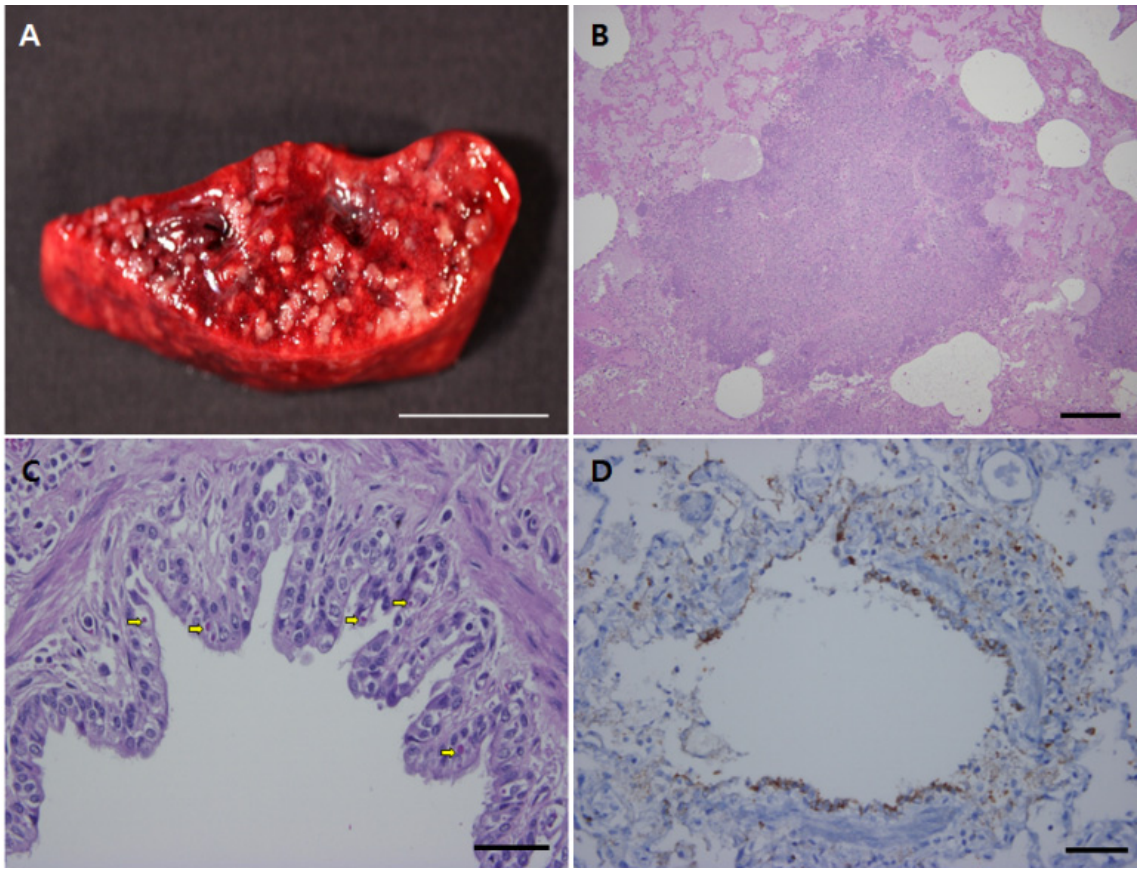

Fig. 1. Gross and microscopic findings in the lung of three badgers. The mottled dark red spots and yellowish nodules on the surface (A). Bar=2 cm. Severe suppurative bronchopneumonia (B). HE. Bar=200 μm. Acidophilic intracytoplasmic inclusion bodies in the bronchial epithelial cells (arrows) (C). HE. Bar=50 μm. CDV antigens in bronchial epithelial cells (D). IHC. Bar=50 μm.

Histopathologic examination revealed a variety of lesions related to CDV infection. Significant histological changes were observed in the lung. The lung showed suppurative bronchopneumonia with multifocal abscesses (Fig. 1B). Additional findings were multifocal necrosis with cell debris and hemorrhages in the alveolar lumen. Many intracytoplasmic inclusion bodies in the bronchial epithelial cells (Fig. 1C) and alveolar epithelial cells were observed. Those inclusion bodies were also found in the epithelial cells of the brain, spleen, liver, kidney, stomach, and urinary bladder. Further, splenic alteration was manifested as severe lymphocytic depletion and liver was observed fatty change and lymphohistiocytic hepatitis with multifocal necrosis.

Immunohistochemically, CDV antigen was strongly detected in the bronchial epithelial cells, which frequently also contained acidophilic intranuclear or intracytoplasmic bodies (Fig. 1D) and macrophages in the alveolar lumen. In addition to the lung, CDV antigens were easily identified in the ependymal cells of the brain, epithelial cells of white and red pulp of the spleen, biliary tract of the liver, biliary tract of the liver, collecting tubule of the kidney, mucosa of the stomach, and the transitional cells of the urinary bladder with various of levels (Table 1). The other organs showed negative reaction in three badgers.

Dense clusters of Gram-positive bacteria were observed in selected sections of the lung tissues of 3 badgers (Fig. 2). For the identification of the bacteria, lung tissue samples from all badgers were collected aseptically, inoculated on blood agar and incubated 24 hours at 37℃ in aerobic condition. The β-hemolytic colonies in all samples were uniformly cultured on blood agar and these isolates were identified as *S. canis* by VITEK II (BioMerieux, Hazelwood, MO, France).

DISCUSSION

Based on the histopathological features and bacterial examination, this case was diagnosed as a mixed infection with CDV and *S. canis*. Although CDV infection have commonly reported in Mustelids (Deem et al, 2000), there are only two case of complex infection as a consequence of CDV-induced immunosuppression in Yellow-throated Marten (*Martes flavigula koreana*) and badger (*Meles meles*) (Hammer et al, 2004; Park et al, 2016).

In the present case, significant features of three badgers were severe bronchopneumonia with multifocal abscess and the presence of both intracytoplasmic and intranuclear inclusion bodies in the epithelial cells of many organs. This findings were consistent with previous reports of CDV infection in other species (Headley and Sukura, 2009; Cho et al, 2015) and badgers (Kim et al, 1997). None of three badgers showed the characteristic hyperkeratosis of the footpad or nose often asso-

Table 1. Immunohistochemical results for antigen detection of canine distemper virus in three badgers

Organ	Immunohistochemical reactivity*		
	Badger 1	Badger 2	Badger 3
Brain	+	-	+
Kidney	++	+++	+++
Liver	++	+++	-
Lung	+++	++	+++
Spleen	+++	+++	+++
Stomach	+	+	+++
Urinary bladder	NT	+++	++

*The immunohistochemical reactivity was measured under microscopy (×400). and classified according to the number of CDV antigen-containing cells observed per slide as follows; -, no antigen detected; +, mild (＜10%); ++, moderate (10～50%); +++, severe (＞50%), NT, not tested.

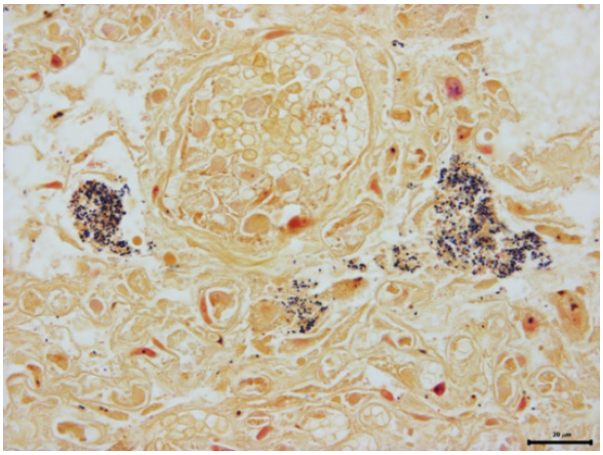

Fig. 2. Gram staining of the lung. Dense clusters of gram-positive bacteria was observed in parenchyma of lung. Gram stain. Bar=20 μm.

ciated with CDV infection.

CDV have been commonly reported to infection of the central nervous system (CNS) leading to a variety of neuological signs (Meertens et al, 2003; Amude et al, 2006; Aguiar et al, 2012). In a previous cases, CDV infected carnivores showed neuological signs such as seizure, tremor and histopathological alteration including demyelination and encephalitis with inclusion bodies in the CNS (Liang et al, 2007; Chen et al, 2008; Headley et al, 2009). Fischer (1965) described lesions of the central nervous system in four free-ranging badgers from Switzerland. In the present case, CDV three badgers had no neurological clinical signs before death and neuropathologic lesions. In addition, unlike the strong immunopositivitiy in other organs such as lungs, liver, and kidney, there were negative or mild reaction in the brain of three badgers.

Canine distemper is a highly contagious disease that can be easily spread between unvaccinated animals (Perrone et al, 2010). In this case, badgers were immunologically naive, so it may have been easily infected from stray dogs around the farm. Further, the 120 badgers that had the similar clinical signs on the farm almost died, suggesting that CDV inflow from outside may have affected high morbidity rated in populated area such as that farm.

In the present case, *S. canis* was also isolated in the lungs of three badgers. The observed lymhopenia and lymphoid depletion in spleen is the evidence of immunosuppressive effects of CDV, suggesting that badgers infected with CDV were more susceptible to develop the secondary infection by *S. canis*. On the basis of the severe necrotic lesions and histopathologic features, there was strong viral replication in the lungs and *S. canis* seem to have been affected to severity of bronchopneumonia with necrosis and abscesses. Aguiar et al. (2012) and Munson et al. (2008) reported that bacterial coinfections impaired immune function and worsen clinical symptoms in CDV-infected animals. Coinfections may affect morbidity and mortality (Goller et al, 2010).

Recently, it has been reported that CDV infection affects not only wildlife but also aquatic mammals and the seriousness of the disease is also shown in farm-reared wildlife (Deem et al, 2002; Sliki et al, 2002; Perrone et al, 2010). Therefore, it is important to investigate the prevalence of CDV in free-ranging carnivore species and surveillance to susceptible infectious diseases that can have a large impact on the animal of interest. Further, early diagnosis of infection is important to quarantine the suspectible CDV infection animals. The immunohistochemical demonstration of CDV antigen proved to be a reliable diagnostic methods (An et al, 2008). In agreement with present reports, IHC examination of biopsy samples from the lungs, liver, and kidney could be recommended for diagnosis of CDV infection. However, compared to IHC, reverse transcriptase polymerase chain reaction (RT-PCR) is the preferred method to early diagnosis for CDV infection because of its sensitivity and specificity (Cho and Park, 2005; An et al, 2008). Therefore, molecular methods are additionally needed for diagnosis of CD cases. We also emphasize to take measures to prevent or reduce secondary proliferation of bacteria in reared farm.

ACKNOWLEDGEMENTS

This project was supported by a grant (Project code no. N-1543069-2015-99-01) from the Animal and Plants Quarantine Agency (APQA), the Ministry of Agriculture, Food and Rural Affairs (MAFRA), Republic of Korea.

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