



Difference Analysis Between Canine Adenovirus Types 1 And 2

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Canine adenoviruses (CAAdVs) include type 1 (CAAdV-1, virulent strain) and type 2 (CAAdV-2, attenuated strain). In recent years, the incidences of CAAdV infections are increasing. However, they are difficult to distinguish when the symptoms are untypical. It is pivotal to find the differences between the two virus types for scientific, epidemiological, and specific treatment. CAAdV-1 (virulent strain) and CAAdV-2 (attenuated strain) induced canine hepatitis (ICH) and tracheobronchitis (ITB), respectively, but the clinical symptom is not obvious. CAAdV-1 and CAAdV-2 have the same genome structure, diameter, morphological features, and cytopathic features, but the same character hinder the diagnose time of the serotypes. CAAdV-1 and CAAdV-2 have a difference in the genome sequence, coding proteins, viral activity, hemagglutination patterns. After infection, pathogenicity and transmission route are different between the two serotypes. Sequence alignment, PCR, Real time-PCR assay are useful methods to distinguish the two serotypes. The attenuated live CAAdV-2 vaccine is currently used to protect against CAAdV-1, but it also has a risk. The further research should focus on the pathogenicity mechanism and the useful vaccine for the two serotypes of canine adenovirus.

Keywords: infection characteristics, distinguishing methods, canine adenovirus 1, canine adenovirus 2, virus genome

INTRODUCTION

Canine adenovirus (CAAdV) belongs to the *Adenoviridae* family and *Mastadenovirus* genus (Decaro et al., 2008). CAAdV is classified into canine adenovirus type 1 (CAAdV-1) and type 2 (CAAdV-2) serotypes (Decaro et al., 2008). Infectious canine hepatitis (ICH) induced by CAAdV-1 is characterized by acute hepatitis. Infectious tracheobronchitis (ITB) induced by CAAdV-2 is characterized by respiratory symptoms (Balboni et al., 2015). There is a high frequency of CAAdV-1 and CAAdV-2 coinfection (Balboni et al., 2014; Crespo et al., 2019; Headley et al., 2019a). Thus, the diagnosis and distinction between CAAdV-1 and CAAdV-2 are important for scientific, epidemiological, and specific treatment in domestic dogs and wildlife.

The differences between CAAdV-1 and CAAdV-2 have been reviewed by Marusyk (Marusyk et al., 1970; Marusyk, 1972; Marusyk and Hammarskjöld, 1972), Hamelin (Hamelin et al., 1984), Zhong (Zhong and Yang, 1990), Linné (Linné, 1992) and Decaro (Decaro et al., 2008). Accordingly, it is necessary to update the currently available literature on the differences between CAAdV-1 and CAAdV-2 as the related research has been developed for nearly 20 years. Clear differentiation between

CAdV-1 and CAdV-2 will help appropriate diagnosis and may prevent the further spreading of CAdVs among domestic dogs and wildlife. Therefore, the virus genome, viral activity, infection characteristic, distinguishing method and vaccination development of CAdV-1 and CAdV-2 are evaluated.

VIRUS GENOME

The whole genome of CAdV-1 and CAdV-2 have been uploaded to the GeneBank (Shibata et al., 1989; Morrison et al., 1997; Zhu et al., 2021). The genetic evolution tree of the virus is shown in **Figure 1**. The genome maps of CAdV-1 and CAdV-2 are showed in the **Figure 2**. The Inverted terminal repeats (ITR) are vital in the replication of the CAdV-1 and CAdV-2, and the ITR length of CAdV-1 (AC_000003.1, 161bp) and CAdV-2 (M17111.1, 196 bp) are different. Cell transformation is a multistep process regulated by the cooperation of several adenoviral gene products encoded in the early regions 1 (E1) and 4 (E4) (Ip and Dobner, 2020). The E1 can participate in cell transformation and promote/inhibit the expression of cellular and viral genes. The E1 region of CAdV-2 has the complementary function of complete cell transformation, but that of CAdV-1 does not have the function (Shibata et al., 1989). The homology between the E1 region of CAdV-1 and CAdV-2 is 75% (Spibey et al., 1989). The region between E1 to the end coding region of the first protein in E1B is 153 bp longer in CAdV-2 than that in CAdV-1. E3 is a nonessential region for adenovirus replication, but E3 gene products play an important role in inhibiting host antiviral immune defense in vivo. Genes on both ends (fiber and pVIII) of the E3 region of CAdV-1 and CAdV-2 show high homology. The E3 region of CAdV-2 (U77082.1, 31323) is approximately 500 bp longer than that of CAdV-1 (AC_000003.1, 30536bp). The 500 bp insertion of the E3 region contributes to the observed biological differences between Cav-1 and Cav-2. CAdV-1 infection was found in the Eurasian wolf (*Canis lupus lupus*) with CAdV-2 vaccine in a French zoological park (Dowgier et al., 2018). The present of CAdV-1 infection suddenly occurs without epidemiology evidence, and it indicates that the sequential circulation of canine adenoviruses 1 and 2 may occur.

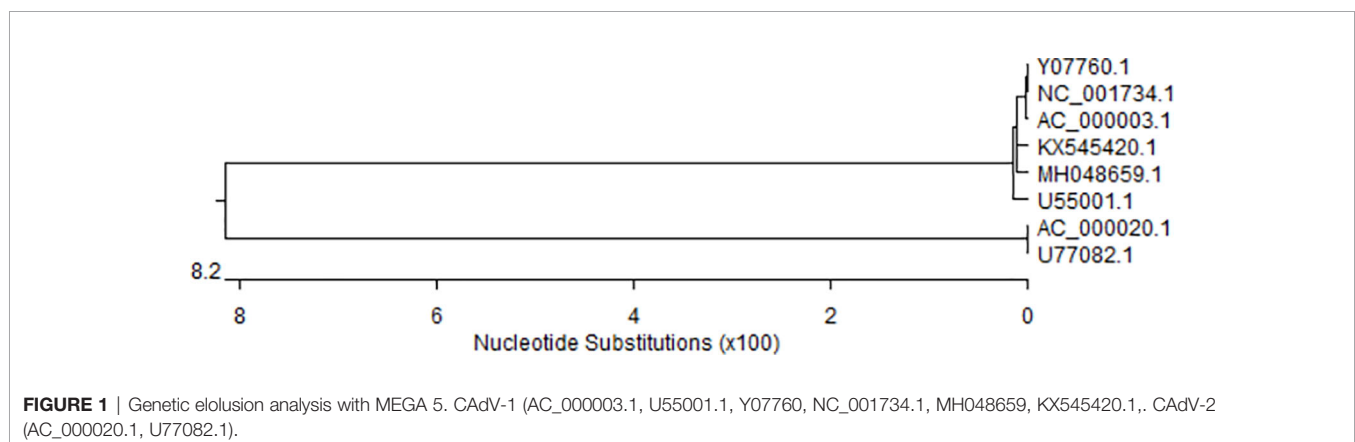
Overall, 75% homology is found, and the sequence alignment can be used to distinguish CAdV-1 and CAdV-2.

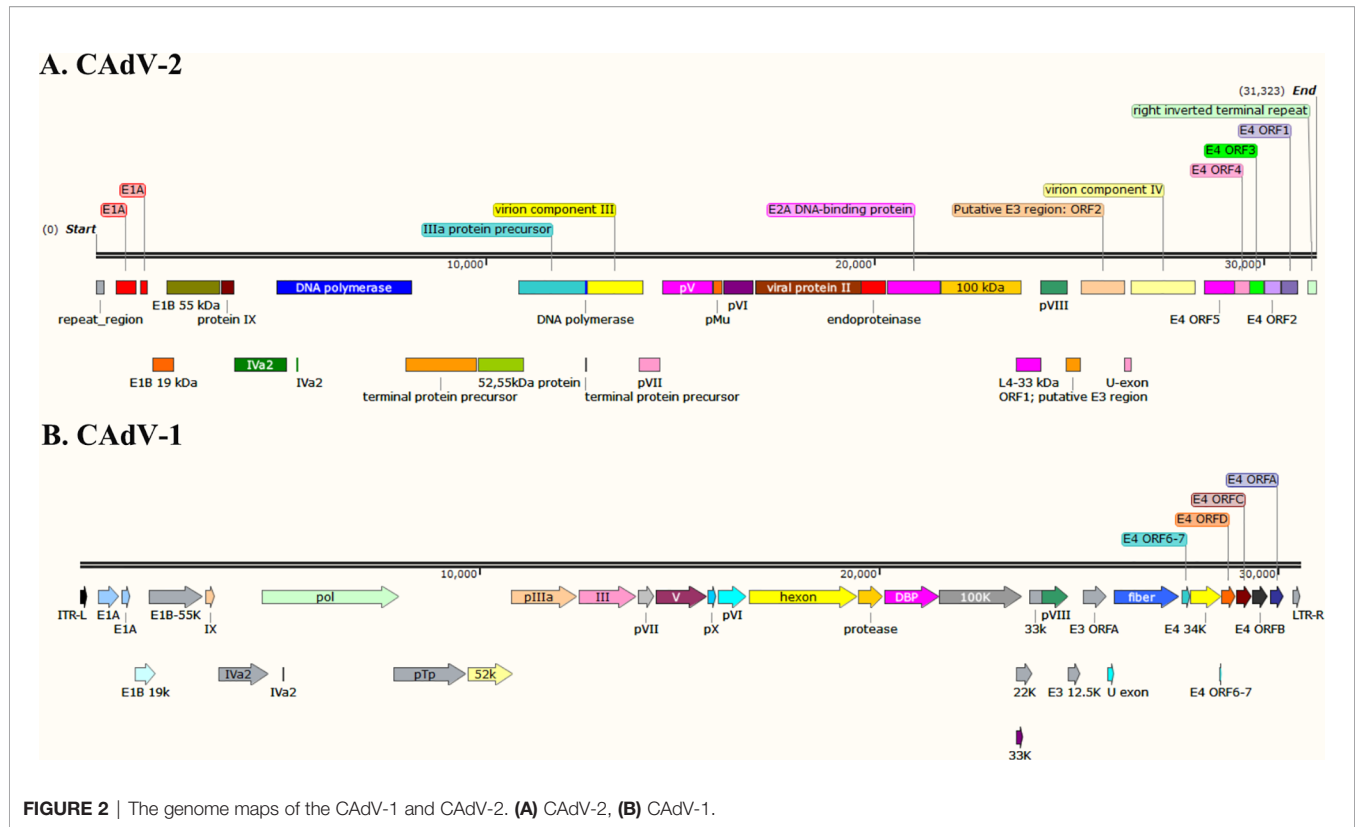
VIRUS ACTIVITY

Viral Structure

CAdV-1 and CAdV-2 have typical morphological features when observed under an electron microscope. The diameter of CAdV is approximately about 70-90 nm, and the shape is icosahedral (Ramidi et al., 2019). The linear double-stranded DNA is wrapped in a protein shell (Verin et al., 2019). The capsid consists of 252 shell grains, of which 240 are hexons, which make up the surface of the icosahedron. The other 12 are penton bases, which are located on the top of the icosahedron. Each has a fiber extending outwards, and the top of the fiber has a ball, namely the Knob. The Knob has the function of adsorbing cells and agglutinating erythrocytes (Yang et al., 2019a). The sialic acid has a binding site on the top of the CAdV-2 knob (Seiradake et al., 2009b). Crystal structures of CAdVs have been reported (Seiradake et al., 2009a), and the three-dimensional structure of CAdV-2 capsid has been constructed. The capsid is responsible for tissue tropism, and the capsid of the CAdV-2 is modified to alter tissue tropism (from canine to human) compared with human adenovirus (hAdV)-5 (Schoehn et al., 2008). However, the three-dimensional structure of CAdV-1 capsid has not been constructed.

Both CAdV-1 and CAdV-2 contain soluble components such as hexon and penton base (Zhong and Yang, 1990). Moreover, hexon is found to carry the complement binding reaction antigen common to the mammalian adenovirus group, and penton base is found to be a completely soluble hemagglutinin (Zhong and Yang, 1990). However, the binding characteristics of the two serotypes for hexon are significantly different because of the structural difference of hexon (Zhong and Yang, 1990). The fiber diameter of CAdV-2 is approximately 35 ~ 37 nm, and it is 10 nm longer than that of CAdV-1. The fiber plays a decisive role in the whole process of the binding of the adenovirus to a host cell receptor. Further, 22 and 40.7 kDa polypeptide are encoded by the E3 regions of CAdV-1 and CAdV-2, respectively





(Linné, 1992; Qin, 2013). These two polypeptides share conserved amino and carboxyl domains, and show conservation at the nucleotide level.

Therefore, they share the same crystal structures and soluble components in the icosahedral. They have differences in the genome structure, diameter, morphological features, encoding proteins (knob, fiber, hexon, and E3 protein) (Figure 2).

Culture Characteristics

The two serotypes of CAAdVs have frequently been proliferated in MDCK cells (Carinhas et al., 2016; Pizzurro et al., 2017). The CAAdV-1 can grow and proliferate in the testicular, lung, liver, and spleen cells of dogs. CAAdV-1 can also proliferate in cells of other members of the canine family, such as raccoon dog kidney cells. The cells of other animals far related to dogs are also sensitive to CAAdV-1, e.g., as the primary cells of pigs, guinea pigs, hamsters, minks, and other animals (Zhong and Yang, 1990). In contrast, CAAdV-2 can only infect tissue cells from the dogs (Fan et al., 2009). CAAdV-2 is not sensitive to primary or passage cells of other animals (such as humans, sheep, monkeys) (Fu et al., 2004).

They show the same cytopathogenic effects (grape-like cytopathic changes) on cell culture (Fan et al., 2009; Yang et al., 2010). However, the viral arrangement in the infected cell is different. CAAdV-2 is closely arranged in a typical crystalline structure in the nucleus of the infected cells (Fan et al., 2009). The arrangement of the CAAdV-1 is relatively loose, and the crystals formed are not as obvious as those seen in

CAAdV-2 (Yang et al., 2010). Further, CAAdV-1 releases progeny virus through nuclear membrane degeneration, whereas CAAdV-2 releases progeny virus through nuclear membrane budding (Zhong and Yang, 1990).

Therefore, they are different in the culture cell types.

Hemagglutination and Antigenicity

Luo proposes that the relationship between the two serotypes of CAAdV is mainly a one-way relationship because CAAdV-1 contains more common antigenic determinants compared with CAAdV-2 (Luo et al., 2008). Whetstone et al. have also reported that the two serotypes have cross-reactions (Whetstone, 1988). The serological analysis shows similarities and differences between CAAdV-1 and CAAdV-2. An anti-serum of CAAdV-2 can neutralize the infectivity of a homologous virus (CAAdV-1) and a heterologous virus (CAAdV-2) with the same ability. However, the anti-serum of CAAdV-1 can only neutralize the homologous virus (CAAdV-1). Furthermore, Accordingly, CAAdV types are distinguishable based on the antigenicity. Because CAAdV-1 and CAAdV-2 have cross-protection, live attenuated CAAdV-2 vaccines are often used to protect animals from CAAdVs.

CAAdV-1 and CAAdV-2 show different hemagglutination patterns (Marusyk and Yamamoto, 1971). CAAdV-2 agglutinates chickens, human (O-type), and albino rat erythrocytes, but cannot agglutinate erythrocytes of mice, geese, pigeons, ducks, cattle, sheep, pigs, etc (Fan et al., 2009). In contrast, CAAdV-1 can agglutinate the erythrocytes of humans (O-type), albino rats (Yang et al., 2010), guinea pigs, and chickens (Hongmei Guo and Ruiliang, 2010).

This difference is associated with different fiber structures between the two serotypes. The Knob of the CAAdV has the function of adsorbing and agglutinating erythrocytes (Yang et al., 2019a). Their hemagglutinins bind to different receptors on the surfaces of erythrocytes.

Further, the properties of hemagglutinin receptor complexes are different. CAAdV-2 binding depends on electrostatic interactions, sialic acid-binding, and the coxsackievirus and adenovirus receptor (CAR) on human erythrocytes (Seiradake et al., 2009b). Based on this characteristic, we can distinguish the two types of CAAdVs (Luo et al., 2008). However, it is not a regular detection method for CAAdVs because dogs are commonly vaccinated with live attenuated CAAdV-2 vaccines. The serum antibody of the CAAdV-2 will present in most vaccine dogs and exert hemagglutination patterns. It will perturb the diagnosis of the CAAdV-2 through hemagglutination patterns. Besides, CAAdV-1 and CAAdV-2 coinfection are often observed.

Therefore, CAAdV-1 and CAAdV-2 have cross-reactions and display different hemagglutination patterns. But an unambiguous diagnosis is not possible by hemagglutination test if there is coinfection with CAAdV-1 and -2.

INFECTION CHARACTERISTICS

Pathogenicity

The two serotypes differ in their pathogenicity, and this may be attributed to the fact that CAAdV-1 and CAAdV-2 are virulent and attenuated virus strains, respectively. Thus, CAAdV types are distinguishable by pathogenetic characteristics. The clinical symptoms of CAAdV-1 have been observed in the fennec fox (*Vulpes zerda*) (Choi et al., 2014), red fox (*Vulpes vulpes*) (Walker et al., 2016a), gray fox (*Urocyon cinereoargenteus*) (Gerhold et al., 2007), and arctic fox (*Vulpes lagopus*) (Balboni et al., 2019). However, CAAdV-1 replicates in healthy red foxes (*Vulpes vulpes*) without clinical symptoms (Walker et al., 2016b), but it does not always have to be asymptomatic. The susceptibility of a silver fox to CAAdV-1 is higher than that of the arctic foxes, and that of the arctic foxes is higher than that of dogs (Sun et al., 2019). Based on the recent data (Balboni et al., 2013; Walker et al., 2016b; Hechinger et al., 2017), red foxes show low susceptibility to CAAdV-1, but the typical lesions of CAAdV-1 are rare (Thompson et al., 2010b; Walker et al., 2016a). The rare typical lesions may attribute to the cross-protection conferred by earlier CAAdV-2 infection, leading to milder clinical outcomes (or subclinical course) of CAAdV-1 infections. In general, the potential influence of existing immunity toward CAAdV-2 may also interfere with the clinical outcome of CAAdV-1 infection. The pathogenicity of CAAdV-1 F1301 strain in dogs, arctic foxes, and silver foxes is different (Sun et al., 2019). This indicates that the pathogenicity of CAAdV-1 is different in different fox species.

Infection with CAAdV-1 is also accompanied by other pathogenic infections. Fungal infection is found in a dog coinfecting with CAAdV-1, canine parvovirus type 2 (CPV-2), and *Cladosporium halotolerans* (Headley et al., 2019a). Further, coinfection of *Pasteurella pneumotropica* and CAAdV-1 is

reported in a puppy (Pintore et al., 2016). CPV and CAAdV-1 co-infection has been observed in 51% of wolves as a previous study (Millán et al., 2016). Co-infection with another pathogen can exacerbate CAAdV-1 infection, increasing the mortality rates. The clinical signs of CAAdV-2 infections are rarely apparent. The typical clinical symptom of CAAdV-2 are observed in CPV-2 (Silva et al., 2014), canine distemper virus (Chvala et al., 2007), and *Mycoplasma cynos* (Chvala et al., 2007) infection animals. Co-infection with other viruses and bacterial exacerbates CAAdV-2 infection.

In brief, CAAdV-1 and CAAdV-2 are virulent and attenuated strains, respectively. Co-infection with other pathogens enhances the pathogenicity of the two serotypes of CAAdVs. Further, the pathogenicity of CAAdV-1 from different fox species is different.

The Host Spectrum

The host spectrum of CAAdV-1 is extensive. CAAdV-1 is found in dogs (Chouinard et al., 1998; Balboni et al., 2014), foxes (Gerhold et al., 2007; Balboni et al., 2013; Thompson et al., 2013; Choi et al., 2014), wolves (Millán et al., 2016), raccoons (Hechinger et al., 2017), Eurasian River Otter (*Lutra lutra*) (Park et al., 2007), and coyotes (*Canis latrans*) (Miller et al., 2009), brown bears (*Ursus arctos*) (Ramey et al., 2018). The spectrum is believed to be narrow before, but new findings suggest that these serotypes also have a vast spectrum. Infection with CAAdV-2 has been documented in dogs (Gerhold et al., 2007; Balboni et al., 2014; Millán et al., 2016; Tımurkan et al., 2018; Yang et al., 2019b), Marsican brown bears (*Ursus arctos marsicanus*) (Di Francesco et al., 2015), raccoon (Dowgier et al., 2018), raccoon dogs, dogs, horses, cattle, cats (Yang et al., 2018) and wolves (Millán et al., 2016). Further, CAAdV-2 is present in healthy red foxes, indicating that CAAdV-2 shows subclinical circulation in wild carnivores (Balboni et al., 2013). Other evidence also shows the prevalence of CAAdV-2 in the wildlife of zoological parks, given that animals with CAAdV-2 infection did not show typical clinical symptoms. Another study that conducted a serological evaluation of ill or dead wild animals reported widespread CAAdV-1 exposure in wildlife from different countries (Dowgier et al., 2018).

Wild foxes are considered to be the bridge between domestic animals and wildlife in terms of CAAdV-1 epidemiology. Based on serological studies, CAAdV is found to be circulating in 97% of island foxes (*Urocyon littoralis*) in California, (Garcelon et al., 1992), 88% of grey foxes in California (Riley et al., 2004), and 94.7% of wolves (*Canis lupus*) in Alaska (Stephenson et al., 1982). The seroprevalence of CAAdV in red foxes has been reported to be 19% -64.4% in the UK (Thompson et al., 2010b; Walker et al., 2016b), 3.5% in Germany (Truyen et al., 1998), 59.6% in Scandinavia (Akerstedt et al., 2010), and 23.2% in Australia (Robinson et al., 2005).

The red foxes are a source for transmitting CAAdV-1 infection to other species (Verin et al., 2019a). Canine adenovirus type-2 infections are found in neotropical otters (*Lontra longicaudis*) from southern Brazil (de Mello Zanim Michelazzo et al., 2022). Red foxes have more chances to contact domestic dogs because red foxes are extensively distributed in Europe and have an intrusive behavior (Bateman and Fleming, 2012). This results in

a growing concern regarding the threat of CAAdV infection from livestock to wildlife. In recent days, further evidence is necessary to ascertain transmission in carnivore species susceptible to CAAdV. More attention should be paid to CAAdV infection dynamics in wildlife.

Thus, the host spectrum of CAAdV-1 and CAAdV-2 is extensive. Red foxes, pine martens, and otters, with inappropriate infections, are the sources of CAAdV-1.

Clinical Symptom

CAAdV-1 and CAAdV-2 have different tissue tropism (Buonavoglia and Martella, 2007). CAAdV-1 are identified within the epithelial cells of the liver and lungs (Headley et al., 2019). The CAAdV-2 mainly invades the respiratory tract epithelium and intestinal epithelium (Michelazzo et al., 2020).

CAAdV-1 transmit through feces, saliva, respiratory secretions, and urine, but CAAdV-2 has an oronasal transmission (Decaro et al., 2008). The main manifestations of CAAdV-1 in dogs, coyotes, raccoons, opossum rats, and skunks are hepatitis, and that in foxes and black bears is encephalitis (Fan et al., 1992; Laurenson et al., 1997), but hepatitis has also been well documented as a symptom of infection in foxes (Thompson et al., 2010a). Neurologic signs of CAAdV-1 are observed occasionally in dogs and may be attributed to the damage of the blood vessels in the nervous system (Hornsey et al., 2019). The deposition of immune complexes may induce iridocyclitis (blue eye) and interstitial nephritis after recovery from CAAdV-1 infection (Decaro et al., 2008). CAAdV-1 can also cause simple respiratory diseases (Hornsey et al., 2019). Low titers of CAAdV-2 have been detected in lung tissue samples (Sun et al., 2019). CAAdV-2, however, causes infectious laryngotracheitis, pharyngitis, necrotizing bronchitis, and other respiratory diseases in dogs and foxes (Balboni et al., 2015a). However, fever is rare in infections with CAAdV-2. Once the disease occurs, it is difficult to treat, and the mortality rate is high.

CAAdV-1 and CAAdV-2 induce different clinical symptoms. As CAAdV-1 is also detected in the lung, respiratory symptoms are not included as an index to diagnose CAAdV-1 and CAAdV-2.

Histopathology

The histopathological changes in the Kupffer's cells and hepatocytes of dogs livers with CAAdV-1 include swelling, yellowish color, centrilobular necrosis, neutrophilic and mononuclear cell infiltration, and intranuclear inclusions (Decaro et al., 2008). Hepatocytes with CAAdV-1 are vacuolated, and cell necrosis is rare. Hepatocyte nuclei with CAAdV-1 are also frequently observed to contain large basophilic inclusion bodies (Hornsey et al., 2019). The gallbladder appears thickened and edematous, and edema of the gallbladder wall maintains during infection (Decaro et al., 2008). Further, the kidney shows congestion, hemorrhage, leukocyte infiltration, and histological changes attributed to vascular damage and inflammation (Decaro et al., 2008). After recovery from ICH, interstitial nephritis and iridocyclitis may occur in dogs (Decaro et al., 2008). Congestion and hemorrhagic lesions are also observed in the spleen, lymph nodes, thymus, pancreas, and hemorrhagic enteritis (Hornsey et al., 2019).

On postmortem examination of dogs infected with CAAdV-2, the histological change in the lung show red areas of consolidation necrotizing bronchitis and bronchiolitis obliterans. It attributes to the damage of bronchial epithelial cells and infection of type 2 alveolar cells (Timurkan et al., 2018). CAAdV-2 infection results in significant respiratory illness in dogs (Yang et al., 2019b).

CAAdV-1 and CAAdV-2 are mainly located in the liver and lungs respectively, and they exert different histopathological changes. After infection with CAAdV-1 and CAAdV-2, nuclear inclusions can form in infected cells (Hornsey et al., 2019), and the formation time and the shape and type of inclusion body have their characteristics (Zhong and Yang, 1990). The liver cell swelling, degeneration, interstitial vascular congestion in the lung, and reduction of the spleen lymphocytes are observed in the silver fox, arctic fox, and dog. The positive immunohistochemical staining with CAAdV-1 is observed in the liver and spleen (Sun et al., 2019).

Based on the above-mentioned data, the inclusion bodies are observed in the tissue with CAAdV-1 and CAAdV-2. The main histopathological changes in infections with CAAdV-1 and CAAdV-2 are observed in the liver and lungs, respectively.

DISTINGUISHING METHOD

In a clinical setting, ocular swabs, feces, and urine samples, and kidney, lung, and lymphoid tissues can be collected to isolate CAAdV-1 and CAAdV-2. Traditionally, viral isolation, electron-microscopic observation (Hongmei Guo and Ruiliang, 2010), and serological tests (Walker et al., 2016b) are used to identify CAAdV-1 and CAAdV-2 infections. Interestingly, respiratory secretions, trachea, and lungs can be used for CAAdV-1 isolation, so the collected tissue do not indicate the type of CAAdV infection. Immunofluorescence (IF) test can be used to determine CAAdV in acetone-fixed tissue sections, smears, and cells. Compared with the conventional pathological methods, immunohistochemical (IHC) analysis is considered to be a reliable diagnostic method for CAAdV infections. IHC of CAAdV-2 diagnosis should be conducted with PCR (Yoon et al., 2010). However, the IF and IHC tests are not adequate for distinguishing between the CAAdV-1 and CAAdV-2, because antibodies of CAAdV-1 and CAAdV-2 have cross-reactions with each other. Thus, viral isolation, IF and IHC tests do not differentiate between CAAdV-1 and CAAdV-2. After CAAdV-1 and CAAdV-2 infection, nuclear inclusion body can be observed by hematoxylin-eosin (HE) staining. But HE-staining cannot identify viruses. It merely makes the intracellular inclusion bodies visible, which indicate viral infection and those inclusion bodies can give a hint as to which virus is probably involved (based on the animal involved, staining characteristics and localization of inclusion bodies within the cell), but they do not identify the virus. The recent discrimination methods are established mainly based on sequence difference. However, it is expensive to sequence the genome of the two serotypes of CAAdVs. The genomes of CAAdV-1 and CAAdV-2 can be differentiated by restriction endonuclease analysis (Assaf et al., 1983; Hamelin et al., 1984) and DNA hybridization (Marusyk and Hammarskjöld, 1972). But the

method is complex and requires high purified viral DNA and more time (Decaro et al., 2008).

One pair of common primers is designed and synthesized according to the sequences of the E3 and flanking regions and a polymerase chain reaction (PCR) assay is established to amplify E3 region in CAAdV-1 (508 bp) or CAAdV-2 (1030 bp) (Hu et al., 2001). Furthermore, the common set of primers are used to amplify the fragments of the CAAdVs in the feces and urine samples, respectively (Chaturvedi et al., 2008). This provides a useful way of identifying the nature of CAV infection. A TaqMan RT-PCR is used to determine CAAdV-1 in red foxes (*Vulpes vulpes*) and raccoons (*Procyon lotor*) in Germany (Hechinger et al., 2017). Moreover, to detect and simultaneously differentiate between the two serotypes of CAAdVs, a duplex RT-PCR is established based on TaqMan technology (Dowgier et al., 2016). Compared with the SYBR Green qPCR, a probe-based RT-PCR assay has high specificity. However, a real-time instrument and two specific probes are necessary to detect the different wavelengths of CAAdV-1 and CAAdV-2. Furthermore, probe-based RT-PCR is more expensive compared with SYBR green qPCR because probes are necessary to the primers. And nucleotide mutations will affect the specificity of reaction in probe-based RT-PCR in the hybridization sites of the probes (Balboni et al., 2015).

Melting curve analysis is also used in the SYBR Green RT-PCR for simultaneous detection and differentiation between the two serotypes of CAAdVs (Balboni et al., 2015b). A binding dye is used to intercalates nonspecifically into the double-stranded DNA in SYBR[®] Green methods, whereas the specificity is improved by sequence-specific amplification in the TaqMan[®] approach. Accordingly, the melting curve analysis based on SYBR Green PCR is performed given that the instrumentation is less complex, the method is simple, and the procedure is cost-effective.

During PCR amplification, the double-standard DNA binds to SYBR Green, and SYBR Green is not a specific fluorescent dye. Thus, the product length and the melting curve analysis method ensured the specificity of the SYBR Green assay. Conversely, primers and probes enhance the specificity of TaqMan technology.

Therefore, sequence alignment, PCR, and RT-PCR assay are useful methods to distinguish between the two serotypes of CAAdVs.

VACCINATION DEVELOPMENT

In 1976, CAAdV-1, which is cultured with MDCK, is inactivated to prepare the vaccine (Fishman and Scarnell, 1976). The inactivated CAAdV-1 vaccine has immunogenicity and safety. But the inactivated CAAdV-1 vaccine cannot protect the CAAdV-

2 from the dog, it is eliminated by the market at last. The CAAdV-1 is attenuated in the MDCK by consecutive passage, the early attenuated CAAdV-1 vaccine is prepared based on the attenuated virus strain (Pepevnak et al., 1960). But the attenuated virus strain has the risk of the virulence return. The attenuated CAAdV-2 virus strain (Toronto A26-61) has cross-protection to CAAdV-1 (Bru et al., 2010). The usage of the attenuated live CAAdV-2 vaccine reduces the spread of the CAAdV-1 and CAAdV-2 (Bass et al., 1980). The virus shedding will find after the immunization of the attenuated live CAAdV-2 vaccine, and the virus shedding will lead to the inapparent infection of the fox without CAAdV-2 (Dowgier et al., 2018), and this phenomenon also attracts attention (Decaro et al., 2007). Thus, the attenuated live CAAdV-2 vaccine has a risk in the clinical setting.

Therefore, the attenuated live CAAdV-2 vaccine is currently used to protect against CAAdV-1 whereas a vaccine against CAAdV-1 is difficult to develop due to safety concerns and less cross-protection. However, the attenuated live CAAdV-2 vaccine also has a risk.

CONCLUSION

CAAdV-1 (virulent strain) and CAAdV-2 (attenuated strain) induced ICH and ITB, respectively, but the clinical symptom is not obvious. CAAdV-1 and CAAdV-2 have the same genome structure, diameter, morphological features, and cytopathic features, but the same character hinder the diagnose time of the serotypes. CAAdV-1 and CAAdV-2 have a difference in the genome sequence, coding proteins, viral activity, hemagglutination patterns. After infection, pathogenicity and transmission route are different between the two serotypes. Sequence alignment, PCR, RT-PCR assay are useful methods to distinguish the two serotypes. The attenuated live CAAdV-2 vaccine is currently used to protect against CAAdV-1, but it also has a risk. The further research should focus on the pathogenicity mechanism and the useful vaccine for the two serotypes of canine adenovirus.

AUTHOR CONTRIBUTIONS

YZ wrote the manuscript. JX, SL, and RZ collected the reference. JH, MW and XY review the manuscript. All authors contributed to the article and approved the submitted version.

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