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Asian Journal of Animal and Veterinary Advances

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Asian Journal of Animal and Veterinary Advances 9 (3): 164-176, 2014
ISSN 1683-9919 / DOI: 10.3923/ajava.2014.164.176

Coronavirus Infection in Equines: A Review

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ABSTRACT

Coronaviruses are positive-sense single-stranded ribonucleic acid (RNA) viruses causing a broad spectrum of diseases in domestic and wild animals including poultry and rodents. Based on antigenic and genetic similarities coronaviruses have been subdivided into 3 major antigenic groups. They infect and produce disease in multiple species of animals, human beings (group 1 and 2) and birds (group 3). *Equine coronavirus* (ECV) causes enteritis in foals. Complete genome of first ECV isolate NC99 strain has been recently sequenced. Cytolytic nature of the virus is responsible for occurrence of lesions in the small intestine, thereby causing diarrhea. Demonstration of *Coronavirus* antigens in clinical samples is test of choice for diagnosis. By electron microscopy (negative staining) *Coronavirus* like particles can be identified in fecal samples. *Coronavirus* antigen in fecal samples can be detected by antigen capture enzyme linked immuno-sorbent assay (ELISA). Molecular detection tool like reverse-transcriptase polymerase chain reaction (RT-PCR) has made the diagnosis more accurate. Virus characterization along with genogrouping has become easier these days with the advent of proteomics and phylogenetic studies. Currently, no vaccine is available for ECV. Biosecurity measures if adopted strictly prevent the disease. The present review highlights the salient features of the *Coronavirus* in general with special reference to ECV and the disease it causes in equines, its epidemiology, diagnosis and appropriate prevention and control measures to be adopted. The review would be helpful for understanding the virus/disease in a better way and alleviating economic losses to the equine/stud farm owners.

Key words: *Coronavirus*, ECV, equine, foal, enteritis, epidemiology, diagnosis, prevention, control

INTRODUCTION

Coronaviruses are ribonucleic acid (RNA) viruses causing a wide spectrum of diseases in humans, domestic and wild animals, poultry and rodents. Some of the animal viruses like *Porcine transmissible gastroenteritis virus* (TGEV), *Bovine coronavirus* (BCV), *Avian infectious bronchitis viruses* (IBV) are of high veterinary significance. Coronaviruses cause

disease ranging from mild to severe enteric as well as respiratory or systemic disease; common colds animals including dogs, cats, swine, cattle/bovines, horses and also recognized in chickens, turkeys, mice, rats and rabbits (White and Fenner, 1994; Studdert, 1996; Jamieson *et al*., 1998; Guy *et al*., 2000; Van der Hoek *et al*., 2004; Lai and Holmes, 2001; McIntosh, 2002; Strauss and Strauss, 2002; Ksiazek *et al*., 2003; Saif, 2004a; Brian and Baric, 2005; Weiss and Navas-Martin, 2005; Decaro and Buonavoglia, 2008; Hansa *et al*., 2012a,b, Suresh *et al*., 2012; Assiri *et al*., 2013; Harriman *et al*., 2013; Kumar *et al*., 2013). The upper respiratory tract along with the gastrointestinal tract is mainly infected by the coronaviruses. Humans are infected by 4 to 5 different strains of the virus that are currently known. The significance as well as economic impact of these viruses is hard to assess as they are difficult to grow under the laboratory conditions. This is very much dissimilar to the rhinoviruses. Even pneumonia is caused by the coronaviruses which may be either direct viral pneumonia or a bacterial pneumonia which is of secondary type (De Groot *et al*., 2011). *Coronavirus* got the focus in 2002/2003 when a new *Coronavirus*, probably of animal origin, emerged in the human population in Guangdong Province, People's Republic of China and evoked a rapid outbreak of disease which spread globally, causing a severe respiratory disease (and often diarrhea) referred to as SARS in human beings (Ksiazek *et al*., 2003; Kuiken *et al*., 2003; Peiris *et al*., 2004; Weiss and Navas-Martin, 2005). The emergence of human SARS incited renewed interest in animal coronaviruses as potential agents of direct and indirect zoonoses (Field *et al*., 1996; Sheahan *et al*., 2008; Field, 2009; Rockx *et al*., 2011). The present review highlights the salient features of the *Coronavirus* in general with special reference to *Equine coronavirus* (ECV) which causes enteritis in foals, a disease of economic significance in equines, its epidemiology, diagnosis and appropriate prevention and control measures to be adopted. The review would be helpful for understanding the virus/disease in a better way, designing effective prevention and control strategies and alleviating sufferings and economical losses to the equine/stud farm owners.

ETIOLOGY

Coronaviruses, positive-sense single-stranded ribonucleic acid (RNA) viruses with helical nucleocapsids, are the members of the *Coronavirus* genus of the Coronaviridae family, order Nidovirales. The *Coronavirus* genome RNA molecule is 26 to 32 kb in size. The virions are enveloped and pleomorphic measuring 80-220 nm in diameter. These have club-shaped peplomers measuring approximately 20 nm in length (Siddell, 1995; Murphy, 1996; Lai and Holmes, 2001; Brian and Baric, 2005). The unusually large, club-shaped peplomers projecting from the envelope gives the viral particle an appearance of a solar "Corona" or "Crown" like morphology as observed under the electron microscope, hence the name "*Coronavirus*" was coined in 1968 for these viruses (Tyrrel *et al*., 1968). Virion has two types of prominent spikes: The long spikes consisting of the S (spike) glycoprotein, present on all coronaviruses giving them their characteristic "corona" appearance and the short spikes consisting of the HE (Hemagglutinin-esterase) glycoprotein, present in only some coronaviruses. The spike (S) glycoprotein of bovine coronaviruses is different from the other *Coronavirus* proteins and is more vulnerable to mutations (Wu and Yan, 2005). The S glycoprotein is an important determinant of species specificity, tropism towards tissue and virulence of *Coronavirus*. Both S glycoproteins and the viral envelope must resist degradation by proteases, low and high pH and bile salts for infecting enterocytes (Lai and Holmes, 2001).

Based on antigenic and genetic similarities, coronaviruses have been subdivided into 3 major antigenic groups that are known to infect and produce disease in multiple species of animals, human beings (group 1 and 2) and birds (group 3). Strain 229E of *Human coronavirus* (HCV);

Porcine transmissible gastroenteritis virus (TGEV); Canine *Coronavirus* and Feline infectious peritonitis virus are group 1 members. Strain OC43 of HCV; *Murine hepatitis virus* (MHV), *Porcine hemagglutinating encephalomyelitis virus* (HEV) and *Bovine coronavirus* (BCV) are members of group 2. *Infectious bronchitis virus* (IBV) and *Turkey coronavirus* (TCV) comprise group 3 (Resta *et al*., 1985; Studdert, 1996; Guy *et al*., 2000; Davis *et al*., 2000; Lai and Holmes, 2001; McIntosh, 2002; Smith and Denison, 2012; Strauss and Strauss, 2002; Van der Hoek *et al*., 2004; Woo *et al*., 2012; Smith *et al*., 2013).

Equine coronavirus (ECV) is a member of the group 2 mammalian coronaviruses and is closely related antigenically to bovine *Coronavirus* (BCV) (Imagawa *et al*., 1990; Guy *et al*., 2000). A high degree of identity (89.0-90.1%) was observed between the N protein sequence of NC99 and published sequences of BCV (Mebus and F15 strains) and HCV strain OC43; while only limited identity (<25%) was observed with group 1 and group 3 coronaviruses (Guy *et al*., 2000; Vijgen *et al*., 2005; To *et al*., 2013).

The complete genome sequencing of the first ECV isolate, NC99 strain, revealed viral genome to be 30,992 nucleotides in length, excluding the poly. A tail (Zhang *et al*., 2007). On sequence analysis and genomic characterization of this isolate, eleven Open Reading Frames (ORFs) were identified encoding 2 replicase polyproteins, 5 structural proteins (HE; S and envelope; membrane and nucleocapsid) and 4 accessory proteins (NS2, p4.7, p12.7 and I). The replicase polyproteins are predicted to be proteolytically processed into 16 non-structural proteins (nsp1-16). The ECV nsp3 protein showed considerable amino acid deletions and insertions (Smith *et al*., 2013).

EPIDEMIOLOGY

Coronaviruses cause a variety of primarily gastrointestinal and respiratory diseases and also neurologic and generalized infections in animals and humans. Only 1 species of animal or at the best a small number of closely related species are usually infected by coronaviruses. But the virus that causes SARS can infect both people as well as animals. Most coronaviruses infect only the cells of their natural host species with marked tissue tropism and also a few closely related species (Lai and Holmes, 2001). Virus causes systemic infections and also localized infections in cases of restricted replication like in the epithelial cells of the respiratory or enteric tracts and macrophages. Coronaviruses possess large RNA genomes and exist as quasispecies increasing the possibility of adaptive mutations and interspecies transmission. While intraspecies recombination among coronaviruses may be common in the field, there are only a few recognized cases of interspecies recombination (Vijaykrishna *et al*., 2007; Gaunt *et al*., 2010; Cabeca *et al*., 2013). The *Coronavirus* diversity is a result of the RNA-dependent-RNA polymerase infidelity; homologous RNA recombination at high frequency along with large size of the genome. More number of coronaviruses that are closely related has been observed from distantly related animals due to the increase in the no. of these viruses. This has resulted in interspecies jumping recently and may cause disastrous outbreak of zoonotic diseases. This is especially true with the virus causing SARS and they have been found to jump from animals to human thereby causing disease of severity in human (Woo *et al*., 2009; McIntosh and Peiris, 2009; www.llnl.gov).

Coronaviruses have been recognized as important pathogens in captive or wild ruminants in the United States, including sambar deer (Cervus unicolor), white-tailed deer (WTD; Odocoileus virginianus), waterbuck (Kobus ellipsiprymnus) and elk (Cervus elephus) (Tsunemitsu *et al*., 1995; Majhdi *et al*., 1997). Recently, Hasoksuz *et al*. (2007) reported the first isolation and characterization of a bovine-like *Coronavirus* from a giraffe (Giraffa camelopardalis) in a

wild-animal park in the US. Also, while studying the evolutionary insights into the ecology of coronaviruses Vijaykrishna *et al.* (2007) observed that bats harbor a much wider diversity of coronaviruses than any other animal species and that diverse coronaviruses are endemic in different bat species, which may likely be the natural hosts for all presently known *Coronavirus* lineages with repeated introductions to the animals and occasional establishment in other species.

As the host range of coronaviruses is wide so the diagnosis as well as prevention and control are based on the clinical and epidemiological evidences. As far as the epidemiology is concerned analysis and risk assessment along with local preparedness are mandatory. Analysis of the spatio-temporal distribution of the disease is of utmost importance. Along with this certain other factors viz., detection of clinically affected animals as well as asymptomatic carriers are important. In case of humans, close personal contact as well as various activities like coughing and sneezing are responsible for transmitting the virus whereas in case of horses faeco-oral route is considered as the most common route of infection. Touching the contaminated objects or surfaces are also responsible for spreading the virus (www.cdc.gov).

DISEASE

Coronavirus enteritis has been suspected in foals with diarrhea; however, direct pathogenicity of *Equine coronavirus* (ECV) in Equidae has not been demonstrated (Davis *et al.*, 2000; Van der Hoek *et al.*, 2004; Arguedas, 2007). *Coronavirus* infection may be suspected only after the other etiologic agents of diarrhea in foals have been ruled out. It is supposed that *Coronavirus* infection spreads in horses via fecal-oral transmission, while respiratory and mechanical transmission may also be feasible (Studdert, 1996; Anzai *et al.*, 2001). Using electron microscopy, Biermann *et al.* (1991) reported 10.6% of the feces from foals contained *Coronavirus* like particles. During a survey of diarrheic foals in Britain and Ireland from 1987 to 1989, Browning *et al.* (1991) reported prevalence of *Coronavirus* though very low. There were no confirmative records of ECV isolation from sick horses before the year 2000, when the first isolation and characterization of ECV (isolate NC99) was reported from feces of a 2-week-old diarrheic foal (Guy *et al.*, 2000). ECV NC99 was serially propagated in human rectal adenocarcinoma (HRT-18) cells. Coronaviruses are difficult to isolate and propagate in cell culture. Utilizing negative contrast Electron Microscopy (EM), *Coronavirus* like particles have been observed in fecal samples from healthy and diarrheic foals (Bass and Sharpee, 1975; Durham *et al.*, 1979; Huang *et al.*, 1983; Reed *et al.*, 1983; Traub-Dargtz *et al.*, 1988; Dwyer *et al.*, 1990; Mair *et al.*, 1990; Browning *et al.*, 1991). Concurrent infections with rotavirus and Cryptosporidium have also been reported (Reed *et al.*, 1983; Dwyer *et al.*, 1990; Mair *et al.*, 1990). Davis *et al.* (2000) identified a *Coronavirus* antigenically related to BCV in a 5-day-old foal with enterocolitis. The *Coronavirus* was recognized in intestinal tissues of the foal by immuno histochemistry using BCV-specific monoclonal antibodies and in feces using an antigen-capture enzyme-linked immunosorbent assay (ELISA) intended for BCV detection (Davis *et al.*, 2000; Guy *et al.*, 2000; Chan *et al.*, 2009; Bidokhti *et al.*, 2013). It is suggested that ECV may spread among horses when they are stabled together or during transport, which is consistent with serologic evidence that BCV or its related virus is widely prevalent in horses in Japan (Imagawa *et al.*, 1990; Anzai *et al.*, 2001). Isolation of ECV from the faeces of horses has also been done with the animal having pyrogenic as well as enteric disease (Oue *et al.*, 2011). Mouse hepatitis virus is another *Coronavirus* that has been studied at molecular level both *in vivo* as well as *in vitro* prior to the discovery of ECV and SARS *Coronavirus*. For multiple sclerosis the virus

has been studied as a murine model. In recent time research efforts have been undertaken significantly on elucidating the pathogenesis of the animal *Coronavirus* including ECV especially by the veterinary virologists (Gouilh *et al*., 2011; Woo *et al*., 2012).

PATHOGENESIS AND PATHOLOGY

The viral replication takes place in the cytoplasm of the infected cell that begins immediately after entry of the virus into the cell. After replication of the ribonucleic acid (RNA) genome there is formation of a long polyprotein that attaches all the proteins (De Haan and Rottier, 2005). The virus possesses a non-structural protein (protease) that separates the proteins present in the chain. For the virus this is a form of genetic character that allows it encoding the greatest number of genes in nucleotides that are small in number. A discontinuous RNA synthesis is the hallmark of the *Coronavirus* infection in all vertebrate animals. By locating certain proteins of the virus in the nucleus of the host cell there may be controlling of the synthesis of macromolecular substances in the cell. The host transcription as well as translation patterns may be changed following infection by various coronaviruses (Enjuanes *et al*., 2008; Doucleef, 2012; WHO, 2013).

The structural proteins that have significant role in structure as well as morphogenesis of virion have got significant contribution to spread of the virus *in vivo* in antagonizing response of host cell (Weiss and Leibowitz, 2011). S glycoprotein binds to specific receptors on the apical membranes of enterocytes thereby undergoing a conformational change which is temperature-dependent and receptor-mediated. This leads to fusion of the viral envelope with the host membranes to initiate infection. Coronaviruses affect the mucosa of small intestine producing significant villous atrophy and also the colon that causes a very severe intestinal damage. This leads to death due to subsequent electrolyte disturbances (http://www.merckmanuals.com). Throughout the length of the villi as well as small intestine enterocytes are infected by enteric *Coronavirus* and the cytolytic nature of the virus is responsible for the occurrence of the lesions directly. There is exfoliation and destruction of the epithelial cells that are absorptive in nature and lining the villi of the small intestine. There is marked shortening of villi due to loss of cells that are infected with the virus. In young animals/foals the lesions are most severe (Holmes and Lai, 1996). ECV has been shown to produce cell death via apoptosis in Madin-darby Bovine Kidney (MDBK) cell cultures (Suzuki *et al*., 2008).

DIAGNOSIS

For diagnosis, demonstration of *Coronavirus* antigens in clinical samples is the test of choice (White and Fenner, 1994). In fecal samples, by electron microscopy (negative staining) *Coronavirus* like particles can be identified (Bass and Sharpee, 1975; Huang *et al*., 1983; Reed *et al*., 1983; Biermann *et al*., 1989; Dwyer *et al*., 1990; Mair *et al*., 1990; Guy *et al*., 2000), however, if the viral particles are not present in sufficient numbers then the EM may require thorough searching or may be futile (Durham *et al*., 1979; Davis *et al*., 2000). Due to the cross-reactivity between BCV and ECV, Serum Neutralization Test (SNT) for coronaviruses in horses employing BCV provides presumptive evidence of exposure to ECV (Bass and Sharpee, 1975; Imagawa *et al*., 1990; Davis *et al*., 2000; Guy *et al*., 2000; Anzai *et al*., 2001). In equine sera, the presence of SN antibodies against BCV may be a common finding; therefore, acute and convalescent samples should be examined for observing increasing titer. With suspected ECV infection in horses, convalescent serum samples may be evaluated approximately 10 days after the

onset of disease. Assaying serum antibody titer to BCV and detecting *Coronavirus* antigen in fecal samples by antigen capture ELISA have been recommended for diagnosis of ECV infection (Davis *et al*., 2000).

Neonatal enterocolitis is an economically significant disease for horse breeders. Therefore, it is important to investigate and identify all potential enteric pathogens. Coronaviral enteritis should be considered a differential diagnosis in cases of equine neonatal enterocolitis. In spite of the reports of feasible *Coronavirus* infection in foals and although coronaviruses or *Coronavirus* like particles have been identified in foals and adult horses with enteric disease, the pathogenicity of coronaviruses and their etiologic role in enteric disease of horses remains unclear and it is unlikely that these viruses are responsible for diarrhea outbreaks in equines (Mair *et al*., 1990; Davis *et al*., 2000; Guy *et al*., 2000; Lester, 2003). Additional studies on NC99 isolate may yield important information about the role of coronaviruses in Equine enteric disease (Bass and Sharpee, 1975; Durham *et al*., 1979; Huang *et al*., 1983; Mair *et al*., 1990; Davis *et al*., 2000). Indirect immunofluorescence test as well as reverse-transcriptase polymerase chain reaction (RT-PCR) have been used for characterization of ECV from foals suffering from diarrhea. Molecular tools like nested PCR, real time PCR and polyacrylamide gel electrophoresis (PAGE) that are generally used against human coronaviruses can also be used for the detection of ECV gene fragment (Guy *et al*., 2000; Van Elden *et al*., 2004). For analysis of the viral genome as well as proteome, segmented mitochondrial RNA (mRNA) is synthesized in cells infected with ECV along with further analysis by northern blotting. Further exploration of the relationship between ECV and other coronaviruses present in group 2 coronaviruses can be done by nucleotide sequence and phylogenetic analysis. On the basis of the amino acid sequences of replicase protein the phylogenetic analysis is done. Irrespective of the gene used, phylogenetic analysis clusters the coronaviruses into 3 groups in majority. It has been clearly shown that equine *Coronavirus* falls into the cluster of group 2a coronaviruses. Most close association is found between ECV and BCV as well as HCV (Gonzalez *et al*., 2003; Bosch *et al*., 2005).

PREVENTION AND CONTROL

The affected animals must be isolated from the rest of the animals promptly. The animals that have recovered must not be put under stressful conditions like hard exercise and travel for a long distance; competition for athletics as well as other procedures (Saif and Heckert, 1990; Spalding and Forrester, 1993; www.worldhorsewelfare.org). Such activities may cause the diarrhea to recur or there may shedding of the pathogen. Recovered animals or horses passing soft faeces continuously shed the organisms potentially in the faeces and therefore, they must be handled with extra precaution and care. Wearing of gloves and boots along with protective gowns are mandatory for personnels in the farm while handling the affected animal for avoiding chances of cross contamination. Faeces as well as bedding materials that are contaminated must not be spread in pastures where other animals may come in the contact thereby increasing the chances of consuming the materials potentially. Landfills are usually selected to discharge off the faeces as well as bedding materials. Composting has been found to be effective in killing the infectious pathogens provided the materials for composting reaches the adequate temperature and if for several months the materials remain unused. Removal of the bedding and faecal materials initiates the cleaning of stall. Detergents must be used for scrubbing the walls and floors. Equipments that are used to clean stall must be kept separated (www.who.int). Advancements in virology, immunology, biotechnology and genetic engineering has paved to the development of

vaccines/vaccine candidates against coronaviral infections in animals and humans but currently vaccines are not available against the *Coronavirus* induced diarrhea in equines (Cavanagh, 2003; Saif, 2004b; Du *et al*., 2008; Decaro *et al*., 2009; Yuen *et al*., 2009; Almazan *et al*., 2013). Antibiotics also prove to be ineffective against this virus as they prove to be ineffective in treating the virus. In less than 10% of the cases however antibiotics are required for prevention of secondary bacterial infections from the gut that gets inflammed. Flunixin meglumine (Banamine) along with supportive care are used for treating the affected horse (Ballon, 1993; Barker *et al*., 1993; Gulland, 1996; www.hagyard.com;www.bi-vetmedica.com; www.thehorse.com).

Rapid advances in diagnosis and surveillance (Schmitt and Henderson, 2005; Belak, 2007; Chen *et al*., 2010; Deb and Chakraborty, 2012; Deb *et al*., 2013; Dhama *et al*., 2012, 2013a, 2014), prophylaxis/vaccines (Saif, 2004b; Meeusen *et al*., 2007; Dhama *et al*., 2008, 2013b; Almazan *et al*., 2013) and emerging therapeutics (Wu and Chan, 2006; Dhama *et al*., 2013c, d, e; Mahima *et al*., 2012; Tiwari *et al*., 2014) need to be given due emphasis for tacking coronaviruses in the era of one world one health (Dhama *et al*., 2013f). Along with these, appropriate prevention and control measures including of strict biosecurity and good management practices need special attention to combat coronaviruses in equines, other animals and humans.

CONCLUSION

Coronaviruses are responsible for broad spectrum of diseases in both vertebrate animals as well as birds. As the viruses of this group cause both diseases of respiratory and gastrointestinal tract they are significant for researchers and clinicians as well as diagnosticians and epidemiologists. With the advent in the field of molecular biology and biotechnology it is easier these days to elaborately describe the typical structure of the virion along with various replicative stages the virus use to undergo in cells that it infects. In foals the virus is associated with diarrhea that is of much economic significance. Pathogenesis of the disease is important to understand the gross and histopathological lesions which help in early diagnosis of the disease. The advancement in the field of diagnosis of ECV infection has started at the beginning of the 21st century. With the aid of electron microscopy; serological test like ELISA; northern blotting and molecular tools like RT-PCR the diagnosis of the disease has become easier. The phylogenetic analysis of ECV has added much to the taxonomic classification of the virus. Involvement of multiple etiology in the enteritis in case of horses/foals increase the importance of molecular detection tools. Researchers thus feel that the wide array of molecular tools that are generally used against human coronaviruses (including various versions of PCR) need to be used much frequently against ECV infection to make the diagnosis more accurate and quicker. Special attention is required as far as the prevention and control of the disease in foals is concerned. Effective biosecurity principles must be followed in the farm premises. Prevention of secondary bacterial infection through use of specific antibiotics is a prerequisite for clinicians to treat the disease. No effective vaccine is available at present for prevention of ECV infection even though vaccine candidates have been selected for animal and human coronaviruses. But with the advent and novel advances in the field of vaccinology it is expected that vaccines will be available in near future.

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