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Application of Transmission Electron Microscopy Techniques in the Veterinary Diagnosis of Viral Gastroenteritis in Livestock Animals

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Abstract

Gastroenteritis caused by viruses is considered to be one of the most important diseases in livestock, being the main cause of morbidity and mortality in young animals, culminating in serious economic losses due to costs with prophylaxis and treatment, increased susceptibility of animals to secondary infections, developmental delay and death. Stressful factors may support the onset of illness. Several viral agents can cause gastroenteritis in various animal species. Rotaviruses are considered the main cause of enteric infections in various animals, including humans constituting important zoonosis. Due to genetic diversity and their ability to cross the species barrier, the coronaviruses infect many species. In cattle, they cause “Winter Dysentery” in adult animals and “Neonatal Diarrhea” in newborn calves. In swine, they are responsible for “Transmissible Gastroenteritis” and “Swine Epidemic Diarrhea.” Equines infected with coronavirus also develop severe gastroenteritis. Bovine viral diarrhea (BVD) caused by a flavivirus of the genus *Pestivirus* is related to digestive and reproductive disorders, affecting any productive sector, are it cut, milk or confinement. Transmission electron microscopy is an indispensable tool in the diagnosis of viral gastroenteric infectious diseases. Negative staining is a simple, fast and efficient technique, being ideal for the detection of gastroenteric viruses, being easily visualized. The immunoelectron microscopy (IEM) technique allows increasing the sensitivity of virus detection where low concentrations of virus are aggregated so that they may be more easily seen. The immunolabeling with colloidal gold technique utilizes specific antibodies tagged with particles of colloidal gold to label the antigen antibody reaction. Embedding resin technique allows obtaining information on the virus–cell interaction. The different transmission electron microscopy modalities promotes a fast and accurate diagnosis of the different gastroenteric viral agents, allowing prophylactic measures of control and prevention in the creations to be promptly instituted, avoiding animal losses and disastrous economic losses, and collaborating with the National Porcine and Bovine Agribusiness.

Keywords: viral gastroenteritis, livestock animals, veterinary diagnostic, transmission electron microscopy

1. Introduction

Gastroenteritis caused by viruses is considered one of the most important diseases in livestock, being the main cause of morbidity and mortality in neonates. The food animal livestock industry estimated a multimillion dollar annual economic loss due to diarrheal diseases associated with a reduction in weight gain, costs with prophylaxis and treatment, increased susceptibility of animals to secondary infections, developmental delay and death of young animals. They represent an important sanitary problem compromising the herds, independently of the level of technification of the creation. Stressful factors such as long-distance travel, reproduction, nutritional deficiencies, environmental changes, etc., may support the onset of illness [1].

The main agents that can cause gastroenteritis in livestock animals are rotavirus, coronavirus and flavivirus.

1.1. Rotavirus

Rotaviruses are considered the main cause of enteric infections in cattle, swine, equines, canines, felines, birds and wild, including humans constituting important zoonosis [2, 3]. They are observed more frequently in neonates, with negative economic impact to the worldwide productive sector, causing high mortality, when it occurs in commercial creations. In livestock, rotaviruses are associated with severe enteric diseases in young calves [4, 5], weaning and postweaning piglets [6] and severe enteritis in foals [7].

Rotavirus is classified as a member of *Reoviridae* family, *Sedovirinae* subfamily and *Rotavirus* genus [8]. They have icosahedral symmetry and a nonenveloped capsid formed by three concentric layers of protein that is 70–90 in diameter. The genome of rotavirus comprises 11 segments of double-strand RNA of 16–21 kbp, encoding six structural proteins (VP1–VP4, VP6 and VP7) and five nonstructural proteins (NSp1–NSp5/6). They are classified into eight groups (A–H) based on the antigenic relationship of its VP6 protein. The most common groups that infect humans and animals are the A, B and C [9, 10].

1.1.1. Bovine rotavirus

Bovine group A rotavirus (bovine RVA) is recognized as the most common cause of severe gastroenteritis in cattle, causing significant economic loss in the dairy and beef industry due to increased morbidity and mortality, treatment costs and reduced growth rates [11, 12].

Infection with bovine rotavirus group A (RVA) has been reported in several countries, such as Brazil [12], the Netherlands [13], Australia [14], EUA [15], New Zealand [16] and Japan [17]. Rotavirus B strains also cause epidemic and sporadic cases of diarrhea in humans, pigs, cattle, limbs and rats [18]. Bovine RVB infections have been reported only in Japan [19], Indian [20], the United Kingdom [21] and the United States [22].

Rotaviral diarrhea usually affects calves between 4 days and 3 weeks old. The incubation period is 12–24 h, and the duration of diarrhea lasts from 2 to 5 days [19, 23–25].

The animals presented depression, anorexia, excessive salivation, profuse diarrhea and severe dehydration [23, 24]. The abomasums typically contains milk curd and thick saliva. The virus replicates in the mature epithelial cells of the villi, and viral infection redirects the function of cells of the absorption for the partially digested milk and accumulates in the intestinal lumen [24, 26]. Other secondary agents often found in epizootics of rotavirus-associated diarrhea may contribute to the severity of the disease [26].

Transmission generally occurs when an unaffected animal has oral contact with infected feces and contaminated feed, or if they are exposed to living quarters with poor hygiene characteristics. Cows displaying signs and symptoms may shed the virus for as long as a week, while some cows can become reinfected and shed the virus throughout their life and remain asymptomatic [26].

The methods of negative staining (rapid preparation) (**Figure 1**) and the immunoelectron microscopy (**Figure 2**) demonstrate the presence of rotavirus particles in fecal samples from calves with diarrhea [25], being named “the gold standard” in the diagnosis of viral enteritis in calves. Immune electron microscopy (**Figure 2**) and immunolabeling with colloidal gold particles (**Figure 3**) have a high sensitivity ranging from 87 to 100% of the different viral agents, which gives good diagnostic value [27, 28].

Figure 1. Negatively-stained rotavirus particles, showing individual capsomers (arrow) in feces of bovine. Bar: 80 nm.

Figure 2. Immunoelectron microscopy of rotavirus particles aggregated by antigen–antibody interaction in bovine feces. Observe “complete” (big arrow) and “empty” (minor arrow) particles. Bar: 240 nm.

Figure 3. Bovine rotavirus marked by the particles of colloidal gold (arrow). Bar: 100 nm.

General recommendations regarding decrease of RVA diarrhea include management practices, especially good hygiene and sanitation procedures, as well as pathogen-specific interventions, such as the use of vaccine prophylaxis [14, 29].

1.1.2. *Swine rotavirus*

In pig farms, rotaviruses are responsible for economic losses due to death of animals, poor growth performance and costs of diagnostic and treatment [30, 31].

The rotaviruses that affect pigs are differentiated as group A, B and C, based on the antigenicity and genetic characteristics of VP6 [9, 32]. Group H has already been described in Brazil [33]. Group A rotavirus is the most frequently isolated of piglets with 1- to 8-week-old diarrhea [34], but groups B and C are also described in piglets in both the maternity and nursery phases [35, 36].

Swine rotavirus has a worldwide distribution and has been reported in Africa [37], Vietnam [38], England [39], Italy [40] and Brazil [33, 41].

Since rotavirus can survive in the environment for an extended period and is transmitted via the fecal-oral route, outbreaks are difficult to control [42].

It affects piglets from the first to the sixth week of life, but occurs with a higher prevalence among animals from 2 to 4 weeks. Infection in neonates is associated with failure of passive immunity due to insufficient colostrum intake or the occurrence of a genotype different from that which occurs endemically in the herd [41, 43].

In pigs, the infection is characterized by vomiting, anorexia, slimming, prostration, diarrhea of liquid or pasty consistency and whitish coloration, which lasts for 2–5 days, and dehydration. In more severe cases, because of episodes of diarrhea, the animal may develop electrolyte imbalance, metabolic acidosis and death [43].

Rotavirus infects enterocytes from the apical and intermediate portions of the intestinal villi, causing lysis of enterocytes with decreased absorption capacity and digestive functions [44, 45].

Both asymptomatic animals and matrices, especially in the period of peri-parto, eliminate rotavirus in the environment and can be considered sources of infection [43].

Rotavirus particles are easily visualized by electron microscopy techniques (negative staining (**Figure 4**) and immune electron microscopy), considering that they are present in large quantities in feces and intestinal fragments of infected pigs. These techniques have been used in many studies of the swine rotavirus [46–48].

Inadequate management practices tend to increase the frequency of rotavirus diarrhea. These include weaning in younger animals, breeding pigs at multiple sites, and larger herds have a higher incidence of RV infection due to variation in the immunity level of females [49].

Delivery assistance and breastfeeding management are essential for newborn piglets to receive passive neutralizing antibodies through the ingestion of colostrum, which is the main form of protection of the newly born piglet against rotavirus.

Figure 4. Negatively-stained rotavirus, showing “complete” (big arrow) and “empty” (minor arrow) particles in feces of swine. Bar: 150 nm.

As with all viruses, there is no specific treatment for rotavirus. Treatment consists of controlling the progression of the clinical picture with the use of supportive therapy (electrolyte replacement) and of the secondary bacterial infections with the use of broad-spectrum antimicrobials [43].

1.1.3. *Equine rotavirus*

Group A equine rotavirus (RVA)-associated diarrhea foals represent a main sanitary problem for the equine industry worldwide [50]. It is the main cause of diarrhea in foals up to 3 months of age, and the acute dehydration can incur severe economic burden due to morbidity in studs [51]. Diarrhea associated with rotavirus can also be a serious problem in areas of intensive breeding during the breeding season [52]. The G3P and P14P are the most prevalent equine rotavirus strain [53].

Clinical signs include diarrhea referred to as white diarrhea or milk diarrhea, lethargy, pyrexia, reluctance to suckle and abdominal tympani. Depression and colic are often observed in serious field cases [51, 54–56]. The malabsorptive watery diarrhea leads to severe dehydration and sometimes death, mainly in neonates with failure of passive antibody transference [50].

Transmission is by feco-oral route via contaminated feces or fomites, and the incubation period is of 1–2 days [57]. The virus invades the intestinal epithelium on the sides and the tips of the villi. The brush border epithelium of the small intestine synthesizes disaccharides to monosaccharides, which are absorbed in the gut. Destruction of the brush border villi results in a decrease in the formation of lactase, resulting in the absence of lactose digestion. This sugar remains in the lumen of the gut, osmotically attracting more fluid [58].

Treatment of foals with rotavirus diarrhea are directed to maintenance of hydration and electrolyte and acid-base balance, aiming to reduce abdominal discomfort or intestinal irritation, to prevent secondary bacterial infection and to avoid spread infection to other foals. The

administration of oral or intravenous electrolytic solutions, in addition to intestinal protectors, has been indicated as an auxiliary method in the treatment of rotavirus diarrhea. As prevention measures, isolation of foals with diarrhea, use of protective clothing for the handlers, hand hygiene, use of pedilavium, and appropriate vaccination of animals should be adopted [59, 60].

Direct electron microscopy readily detects rotavirus particles in feces and intestinal fragments of foals with diarrhea [61–64].

1.1.4. *Sheep and goats rotavirus*

Rotaviruses A, B and C have been described in small ruminants [65–67]. Reports on ovine or caprine Rotavirus A are available from various countries worldwide, with detection rates reaching 60% and estimated 10–30% mortality [68–70].

During outbreaks of neonatal diarrhea by rotavirus A, prevalence in fecal samples and lamb, morbidity/mortality may be very high [71, 72]. During an outbreak of diarrhea occurred in a dairy herd of goats in Brazil, rotavirus A was detected in 80-day-old animals with watery diarrhea, anorexia, dehydration and death of one of the animals [73]. Rotavirus A has been associated with diarrhea in goats kids [66, 74]. Regarding the occurrence of rotavirus C, little information is obtained in these species [74].

Administration of colostrums is pivotal to protect lambs from rotavirus-induced diseases [75]. The colostrums and milk of ewes administered with an inactivated rotavirus A vaccine 2–3 weeks prior to mating contained high titers of antibody to the virus [76].

1.2. Coronavirus

1.2.1. *Bovine coronavirus*

Due to genetic diversity and their ability to cross the species barrier, the coronaviruses infect many animals species, including cattle, pigs, equines, rodents, dogs, cats, ferrets and domestic and wild birds [77].

1.2.1.1. *Bovine enteropathogenic coronavirus*

Bovine coronavirus is widespread in the cattle population, resulting in economic losses to the beef and dairy industry in the world [78]. In both beef and dairy herds, BCoV can be associated with calf diarrhea, calf respiratory disease, winter dysentery, respiratory disease in adult cattle, and combined pneumonia and diarrhea in calves and adults [79, 80].

Bovine coronavirus belongs to the *Nidovirales* order, *Coronaviridae* family and *Betacoronavirus* genus [9]. They are simple-stranded positive sense RNA viruses, 32 kbp long, which associates with the nucleoprotein (N) forming a nucleocapsid with helical symmetry. The viral envelope of BCoV is formed by a lipidic double layer with five structural proteins (M, sM, HE, S and I) [81, 82].

Morphologically, they are pleomorphic, with radial projections with a form like-club giving an aspect of solar corona and they measure 75–160 nm of diameter [83].

Transmission of bovine enteropathogenic coronavirus occurs by the fecal-oral or respiratory routes, and most often transmission is horizontal and occurs from carrier dam to offspring postpartum [84].

BCoV causes severe hemorrhagic diarrhea, which is sometimes fatal in young animals, and the spiral colon is the host spot for viral replication in the gastrointestinal epithelium, leading to intestinal villi atrophy and osmotic diarrhea [85].

Once infected, a calf can secrete high levels of virus within 48 hours after experimental infection, and this may persist up to 14 days [86].

The clinical signs are represented by yellow to blood-stained mucus-containing diarrhea, which then progress to a profuse watery diarrhea. Subsequently the animals become dehydrated, depressed, weak and hypothermic, and their suckle reflex is loosened. Most of calves recover, but a few develop pyrexia, recumbency, coma and death [79].

1.2.1.2. Winter dysentery (BCoV-WD) in adult cattle

Winter dysentery (BCoV-WD) is a sporadic acute, contagious hemorrhagic enterocolitis of cattle that occurs in epizootic fashion in a herd [87].

BCoV-WD has been reported through the world including EUA [88], France [89], Spain [90], Canadian [91], Italy [92], Japan [93] and Brazil [94].

The incubation period for BCoV-WD ranges from 2 to 8 days [79].

The disease is characterized by a sudden onset of mucous dark, watery often-bloody diarrhea, which is accompanied by depression and anorexia in adult beef and dairy cattle. Mild to moderate signs of respiratory disease have been reported [92, 95]. The outbreaks occur during the winter season and result in high morbidity and low mortality rates. In an affected cattle herd, milk production may not return to normal for several weeks or even during that lactation period, resulting in significant economic losses for the milk industries. Cattle are more efficiently infected in winter, which increases the environmental contamination and justifies the high morbidity of winter dysentery during the cold months [96, 97].

The intestinal lesions are comparable with those observed in calves with BCoV-induced diarrhea [79]. In calves with BCoV enteric infection, viral particles can be detected by electron microscopy in the feces 1–2 days before the onset of diarrhea and for several days after the diarrhea has resolved. BCoV can also be found in nasal secretions of calves with BCoV diarrhea. Recovered calves that are apparently immune to disease can still shed BCoV in their nasal secretions or feces [98].

Electron microscopy has been widely used to detect bovine coronavirus particles. Typically, coronavirus particles can be demonstrated in fecal samples by direct electron microscopy (**Figure 5**), immune electron microscopy or immunolabeling with colloidal gold particles (**Figure 6**) [27, 98–102].

In cases of coronavirus infection, the most indicated treatment is the symptomatic with electrolytes, antipyretics, antidiarrheals and probiotics, antimicrobial therapy to prevent secondary

Figure 5. Negatively-stained coronavirus particles containing distinctive club-shaped surface projections, in feces of bovine (arrow). Bar: 100 nm.

Figure 6. Buffalo coronavirus marked by the particles of colloidal gold (arrow). Bar: 100 nm.

infections, and the occurrence of outbreaks to vaccinate the animals. Colostrum intake has emerged as the natural and most useful method to control BCoV calf diarrhea [103]. The hygiene, management and sanity of the property are important factors for the prevention of neonatal diarrhea, thus avoiding serious damage to the producer [104].

1.2.2. Porcine coronavirus

1.2.2.1. Porcine transmissible gastroenteritis (TGEV)

Porcine transmissible gastroenteritis is a highly severe contagious disease caused by virus of the *Coronaviridae* family and genus *Alphacoronavirus* [77].

As a notifiable disease, the TGEV causes significant economic losses in the pig industry and has been reported in several countries as Europe, American and Asia [105–107]. TGEV was detected for the first time in Brazil through histopathological techniques and the transmission electron microscopy in 19 (25.3%) small intestine samples of pigs from various municipalities in the State of São Paulo and the Minas Gerais, Brazil [108].

The disease affects pigs of all ages, and symptoms were represented by severe watery diarrhea accompanied by vomiting [109, 110], anorexia, prostration, dehydration, dyspnea and death [108]. In the TGEV epizooty, the high mortality rates, up to 100%, affecting piglets of less than 2 weeks of age is a result of severe dehydration [105].

The replication of the virus occurs in the digestory and respiratory tracts, and the target is the epithelial cells of the small intestinal villi that result in the atrophy of the infected epithelium focusing in severe intestinal disorders, which can be fatal in the neonatal period [111, 112].

The transmission route occurs by breast feeding, oral-fecal and fomites [113]. Exportation of fresh and frozen pork contaminated by TGEV allows that these types of food act as a potential source of viral transmission [105, 107, 114].

For the diagnosis of swine coronavirus, the negative-staining technique (**Figure 7**) has been widely used by many authors [115–119]. To confirm the viral strain (TGEV), immunoelectron microscopy (IEM) (**Figure 8**) and immunolabeling techniques with gold particles, performed with a monoclonal antibody specific for TGEV, can be used. Viral ultrastructural aspects can be studied through the resin embedding technique (**Figure 9**) [108].

According to the OIE [107], technique of transmission electron microscopy and *in situ* hybridization was chosen to identify TGEV. Using specific monoclonal antibodies it is possible to differentiate TGEV from the coronavirus that causes epidemic porcine diarrhea and the coronavirus that causes respiratory disease.

Figure 7. Negatively-stained coronavirus particles, showing characteristic envelope with radial projections forming a corona, in feces of swine (arrow). Bar: 100 nm.

Figure 8. Immunoelectron microscopy of TGEV (arrow) aggregated by antigen–antibody interaction in swine feces. Bar: 190 nm.

Figure 9. Resin embedding of swine small intestine. Observe coronavirus particles, incomplete, within intracytoplasmic vacuoles (big arrow), complete particle (minor arrow) and dilated cisternae of the rough endoplasmic reticulum (arrow head). Bar: 200 nm.

There is no specific treatment for TGEV. Treatment is symptomatic and seeks to avoid the spread and control of secondary infections, which may aggravate clinical signs. The most important prophylactic measure is to prevent the entry of TGEV in herds [120].

1.2.2.2. *Epidemic porcine diarrhea (PEDV)*

The epidemic porcine diarrhea virus (PEDV) has been causing incalculable losses to the production of pigs in several countries, modifying the behavior of the swine market worldwide [121].

It was first observed in England, causing a devastating and sudden diarrhea during the winter, leading to losses in pig farms [122]. Subsequently, it was found in Belgium, Hungary, France, Italy, the Czech Republic, China and Asia, where more severe outbreaks were diagnosed. In the USA, it was first diagnosed in May 2013, and during the period from September 2013 to February 2014, losses of pigs in the United States by the swine epidemic diarrhea virus were estimated in 2.7 million or slightly more than 5% of the animals [123–126]. Recently, the presence of the virus has been reported in countries of South America, such as Peru [127] and Colombia [128].

The direct transmission occurs by fecal-oral route. Clinical signs of PEDV may occur within 4–5 days following introduction of infected swine to farms with susceptible animals. Following an outbreak, PEDV may subside but may become endemic if sufficient litters are produced overcome lactogenic immunity. Contaminated personnel equipment or other fomites may introduce the virus into a susceptible herd [121].

The incubation period of PEDV is 3–4 days [129].

Clinical signs of PEDV infection include anorexia, vomiting, diarrhea and dehydration. Morbidity and mortality in piglets less than 5 days is of almost 100% due to severe diarrhea and dehydration, but mortality in piglets older than 10 days is about 10% [130, 131].

PEDV replicates in the cytoplasm of villous epithelial cells throughout the small intestine, destroying target enterocytes because of massive necrosis or apoptosis. These processes lead to villous atrophy and vacuolation as well as a marked reduction in the enzymatic activity, causing malabsorptive watery diarrhea, followed by serious and fatal dehydration in piglets [132–134].

Ultrastructural colon lesions have been observed by transmission electron microscopy. At the cellular levels, PEDV protein E is localized in the endoplasmic reticulum with small amounts being found in the nucleus of infected cells [135].

Although an approved vaccine against the PEDV virus is not yet available, it is important to adopt biosecurity programs to prevent the spread of the disease in the country. These include the disinfection of environments susceptible to contamination, such as breeding, slaughtering and transport facilities, as well as water and food containers. It is also necessary to implement surveillance measures regarding the introduction of new animals in the herd, quarantine procedures and control of access to the farms [121].

1.2.3. *Equine coronavirus (ECoV)*

Equine coronavirus (ECoV) which causes enteritis in foals is a disease of economic significance in equines for horse breeders [136].

They typically have a restricted host range, infecting only their natural host and closely related animal species but do have the capacity to cross the species barrier to infect new hosts. Equine coronavirus (ECoV) is the only coronavirus known to infect or cause disease in horses [137].

Equine coronavirus (ECoV) belongs to the *Coronaviridae* family and *Betacoronavirus* genus [138].

ECoV was first isolated in North Carolina (USA) from the feces of a diarrheic foal in 1999 [139] and was initially believed to only affect foals. Since 2010, there have been several reports of ECoV-associated respiratory and enteric infections in adult horses in Japan, Europe and the United States, but its global distribution is still poorly defined [10, 140–143]. In Brazil, the first outbreak of enteritis in horses was reported in 1988 by transmission electron microscopy in a horse livestock, in São Paulo, SP, affecting animals ranging from 1 week to 4 months that presented aqueous diarrhea [144]. Later, another outbreak was reported in a farm in Rio Grande do Sul, where 69 foals of 45–90 days were affected by severe enteritis [145].

The main clinical signs presented by the ECoV infection are represented by anorexia, apathy, lethargy, fever and neurologic abnormalities (ataxia, depression and recumbency). Respiratory problems, profuse aqueous diarrhea greenish to yellowish of putrid odor and discrete algia abdominal can also been observed [136, 141, 144–147]. It is transmitted by the fecal-oral route, and signs tend to resolve in 1–4 days, although animals can continue shedding for several weeks [147]. It is suggested that ECoV may spread among horses when they are stabled together or during transport [148, 149].

The virus has been diagnosed more frequently in adult animals over 2 years of age [146, 147, 150]. However, outbreaks have been reported in foals from 5 days to 4 months of age [139, 144, 145, 151] and in both adults and young animals [141].

Morbidity ranges from about 20–57%, and mortality is typically rare [147]; however, a high mortality rate has been described in foals [152].

ECoV has been shown to produce cell death via apoptosis in Madin-Darby Bovine Kidney (MDBK) cell cultures [153].

With the aid of transmission electron microscopy techniques, the disease is more easily diagnosed. Negative-staining technique (rapid preparation) (**Figure 10**) and immunoelectron microscopy (**Figure 11**) has been widely used for direct visualization of viral particles [139, 144, 146, 151, 154–157].

Most adult horses with clinical ECoV infection recover spontaneously in a few days without specific treatment. Horses with persistent elevated rectal temperature, anorexia and depression are routinely treated with anti-inflammatory drugs intravenously. Horses with colic, persistent depression and anorexia and/or diarrhea have been treated more intensively with fluid and electrolyte until clinical signs have resolved. Additionally, antimicrobials and gastrointestinal protectants should be considered in horses with secondary bacterial infection. The use of BCoV vaccine in horses for the prevention of ECoV has not been investigated and cannot be recommended.

Figure 10. Negatively-stained coronavirus particles (arrow), in small intestine suspension of equine (arrow). Bar: 190 nm.

Figure 11. Immunoelectron microscopy of coronavirus particles (arrow), aggregated by antigen-antibody interaction in equine feces. Bar: 190 nm.

The prevention of ECoV infection should focus on the implementation of routine management practices aimed at reducing the likelihood of introducing and disseminating ECoV at any horse-based premise (boarding facility, show ground, and veterinary hospital). Once an ECoV infection is suspected, strict biosecurity measures including footbaths and the use of personal protective equipment should be provided and adequately maintained for sanitary purposes [158].

1.3. Bovine viral diarrhea (BVD)

Brazil currently occupies the position of the world's largest exporter of beef, with the production chain moving around 167.5 million/year, producing 9.5 million tons [159].

Bovine viral diarrhea virus (BVDV) is an important pathogen of ruminants causing severe economic losses to the cattle industry, affecting any productive sector, be it of cut, milk or confinement [160].

It has a worldwide distribution, having already been reported in several countries, such as Italy [161], Australia [162], EUA [163], China [164] and Japan [165].

In Brazil, the presence of BVDV has already been proven in several states, such as Pernambuco [166], Goiás [167], Maranhão [168], Minas Gerais [169] and Rio Grande do Sul [170].

Bovine viral diarrhea virus type 1 (BVDV-1) belongs to the *Flaviviridae* family and *Pestivirus* genus that comprises also the species bovine viral diarrhea virus type 2 (BVD-2), classical swine fever virus (CSFV) and border disease virus (BVD) [8].

The virions are 40–60 nm in diameter, spherical in shape, and contain a lipid envelope [171]. The *Pestivirus* genome composed of a positive-sense single-stranded RNA that is approximately 12.3 kb [172]. BVDV-1a and 1b are the most widely distributed BVDV-1 subtypes in the world and alternate as the most prevalent in different countries [173, 174]. BVDV-1 can be divided into at least 21 subgenotypes (1a–u). BVDV-1i is an uncommon subtype that has been reported in the United Kingdom and Uruguay, and recently, in Brazil [175, 176], and the subtype 1 h strain was isolated in Italy [175]. Bovine viral diarrhea virus is one of the most widespread cattle pathogens worldwide being considered emergent [177].

The clinical signs of BVDV infection are highly variable, ranging from unapparent or mild infection to fatal acute illness. These clinical signs or symptoms include acute or chronic gastrointestinal disorder, respiratory disease in calves, and a hemorrhagic syndrome with thrombocytopenia, skin diseases, immunosuppression and decreasing milk production. BVDV also had been related with infertility, return to estrus, embryonic or fetal mortality, abortion or mummification, fetal malformations or the birth of weak calves and infeasible [178–180].

The BVDV induces persistent fetal infection, generating a persistently infected animal with 50% calves lethality rate in the first year of life. The PI animals excrete the virus in large quantity in the secretions, being responsible for the maintenance of the virus in the herd [181].

Mucosal disease affects exclusively PI animals between 6 months and 2 years of age, with a fatal course. The acute form is characterized by a 10- to 14-day incubation period, followed by fever, anorexia, tachycardia, polypnea, erosions in the oral mucosa and nostrils, dehydration, watery diarrhea and rhinitis, nasal and ocular discharge and death within a few days. Animals that survive acute form develop chronic mucosal disease, whose signs are nonspecific [181].

Transmission may occur horizontally through direct contact between animals or indirectly through contaminated secretions, excretions and fomites or vertically leading to congenital infection of the fetus [180].

The introduction of BVDV in the herds occurs by the entry of PI animals on the farms, through the acquisition of cattle during the acute phase of the disease, persistently infected bulls or female breeding PI fetuses and contact between neighboring herds [182].

The laboratory diagnosis is performed through seroneutralization, cell culture isolation, PCR and immunohistochemistry [180].

Bovine virus diarrhea (BVD) particles have been identified by negative-staining electron microscopy (**Figure 12**) in feces, in purified virus preparations, in infected cell cultures and in tissues from infected animals [183–188], being this technique recommended by the OIE for the detection of BVDV [183].

Immunogold labeling technique was utilized for marking BVDV particles (**Figure 13**) and for locating both E (rns) and E2 proteins at the virus membrane [187]. The embedding resin technique was used to study the ultrastructural aspects of BVDV, showing that bovine viral diarrhea virus NS4B protein is an integral membrane protein associated with Golgi markers and rearranged host membranes [189].

Figure 12. Negatively-stained flavivirus isometric particles in bovine intestine suspension (arrow). Bar: 70 nm.

Figure 13. BVDV particles strongly enhanced by the dense colloidal gold particles (arrow). Bar: 140 nm.

The examination of suspected sample by immune electron microscopy procedures is feasible for BVDV virus detection, and it can be used as diagnostic tool, especially for screening of cell culture supernatants infected with suspected clinical specimens. The suitability of this assay for direct screening for identification of persistently infected animals, where the viral load is expected to be high and which are of great concern for control programs, needs to be explored [188].

Considering that BVDV infections cause significant economic losses to farms, biosafety, sanitary and hygiene measures should be implemented on farms to reduce the prevalence of the virus in herds. In addition, quarantine, vaccination and testing procedures should be instituted in the herds for identification and removal of persistently infected animals (IP), minimizing the spread of the virus [166, 178, 179, 190].

1.4. Transmission electron microscopy

Transmission electron microscopy is a perfectly adequate tool to investigate viral agents during outbreaks of gastroenteritis [191]. It is used when it is necessary to apply a fast and reliable diagnostic method to contain the infection and to quickly minimize the animal losses and consequently the economic damages that cause to the rational economic exploitation of the production animals, as much by the decrease of the productivity as in terms of treatment costs. Consideration should also be given to the zoonotic potential of some viruses, such as rotavirus, and the implications for public health [1].

Electron microscopy has led to the discovery of many new viruses, mainly those associated with gastroenteritis, for which it remained the principal diagnostic method [192].

Viruses are grouped into families based on their morphology. Viruses from various families look distinctly, and these morphological variances are the basis for identification of viruses by electron microscopy. The identification to the family level is already sufficient for the recognition of an unknown infectious viral agent and, to allow the immediate adoption of prophylactic measures, control and prevention of the disease [193].

1.4.1. Negative-staining technique

Negative staining has been a useful specimen preparation technique for biological and medical electron microscopists for almost 50 years since its introduction by Brenner and Horne in 1959 [194] as an established method [195].

The technique consists of an electron-dense stain that surrounds the biological specimen and penetrates in the structural crevices to give an image in which the biological specimen appears electron-lucent against the dark electron-dense background. The image is formed by the absorption or deflection of the electrons by the stain, giving opacity to those areas [195, 196].

Due to the simplicity of the preparation of the samples and the rapidity of the results (5–10 min), negative staining has been the most used technique, mainly in the detection and viral identification during outbreaks of gastroenteritis [197]. The large diversity of viruses potentially involved in gastroenteritis contributed to the use this technique in clinical virology [198].

In addition to the rapidity, it has several other advantages, such as to enable the detection of different viral particles in a single sample without the need for specific reagents, to allow the discovery of new viruses, and to require a small amount of sample, besides when detecting the agent to exclude the possibility of obtaining false-positive results [193].

Negative staining can be applied to various types of biological samples. In cases of gastroenteritis, viruses can be easily visualized in feces, small intestine fragments, fecal swab and peritoneal fluid where they are found in large quantity.

Several types of contrasting (heavy metal salts) are used; however, 2% ammonium molybdate and pH 5.0 provide the best contrast to viral agents.

The diagnosis is made by comparing the dimensions and specific morphology of the visualized particles and other taxonomically combined viral families.

1.4.2. Immunolectron microscopy technique

Immunolectron microscopy (IEM) technique that consists in the direct visualization of antigen and antibody complexes by negative stain which promotes increased sensitivity in 100-fold, cujo resultado positivo, is indicated by the presence of virus-antibody aggregates [192].

The technique was initially developed to quantify plant by Derrick [199] and was subsequently used in several types of clinical samples [200–202].

Immunolectron microscopy (IEM) is utilized when the number of viral particles in a sample is very low, when virions are pleomorphic and difficult to identify because they do not have a typical viral morphology or when the samples are “dirty” because the aggregated complexes are more easily observed [203]. It allows identification of the virus for specific antigen-antibody reaction and such identification is achieved by its morphology. It is also used to serotype morphologically similar (but antigenically distinct) particles [195, 204, 205].

Several variations of the method such as immune clumping or direct immunolectron microscopy (DIEM) [206, 207] or immune aggregation electron microscopy (IAEM) [203], solid phase immune electron microscopy (SPIEM) [199] and decoration [208] have been used. Hyperimmune sera, monoclonal antibodies or convalescent sera can be used in performing the technique [193, 203]. The SPIEM has been utilized to detect most of the viruses that cause gastroenteritis such as bovine rotavirus, swine, equine, canine, bovine coronavirus, swine, canine parvovirus and BVDV.

IAEM was used to detect porcine rotavirus (PoRV), porcine torovirus (PoToV) and porcine epidemic diarrhea coronavirus (PEDV) in pigs with enteritis utilizing convalescent sera [203].

1.4.3. Immunolabeling with colloidal gold particles by negative-staining technique

In this technique, the antigen-antibody reaction is enhanced by antigen labeling by colloidal gold particles associated with protein A, using type- and genus-specific antibody. The method also allows detection and identification of antigen structures induced by the virus and its localization in infected cells, serotype viral strains [209], and determines antigenic variants in isolated strains [210].

This technique was used to label TGEV particles in feces and small intestine fragments of infected pigs [108], type A rotavirus and coronavirus in samples from diarrheic calves and winter dysenteric cattle [143], the simultaneous presence of coronavirus and rotavirus in feces of calves with diarrhea [211] and BVDV in feces of cattle with diarrhea [184].

1.4.4. Immunolabeling in ultrathin section technique

Immunolabeling in ultrathin sections are powerful tools for detecting and localizing proteins in cell and tissues and to detect virus or viral antigen on the surface of or within ultrathin sections of the cells [195, 212, 213]. The two most widely used techniques are pre-embedding and post-embedding techniques. The pre-embedding method primarily detects determinants exposed at the surface of infected cells such as virus receptors or envelope glycoproteins of budding viruses that are freely accessible to antibodies and reagents. The post-embedding labeling of thin sections allows access to determinants present in the different compartments of the cell and to internal viral structures since they become exposed at the surface of the section [214]. Antibodies coupled to electron-dense markers such as colloidal gold can reveal the localization and distribution of specific antigens in various tissues. The colloidal gold has been the most widely used marker [215].

Immunolabeling in ultrathin sections has been widely applied to elucidate ultrastructural pathological aspects of gastroenteric viruses. Payne et al. [216] studied bovine coronavirus antigen in the host cell plasmalemma in cells traced with colloidal gold particles. Risco et al. [115] investigated the presence of two types of virus-related particles that are found during transmissible gastroenteritis virus (TGEV) morphogenesis, whereas Salanueva et al. [217] reported aspects of the structural maturation of the transmissible gastroenteritis coronavirus (TGEV). This technique has also been applied to check the exploitation of microtubule cytoskeleton and dynein during canine parvoviral traffic toward the nucleus [215].

1.4.5. Resin embedding technique

The resin embedding technique followed by ultrafine sections is especially important to reveal fine details of the ultrastructure of all types of cells and tissues [218], and in an infectious process, it allows observing pathogenesis of infection and the identification of the agent [205]. The thin sectioning has the advantage of allowing the observation of virus cell interaction, which reveals the site of virus replication and maturation in the host cells, a pertinent information in the identification of unknown viruses [219].

The ultrastructural set of details not only determines the infection, but also the course of the disease in the creations [220].

Resin embedding technique allowed to study several ultrastructural aspects of the intracellular behavior of the TGEV in intestinal fragments of infected pigs [108] and of the parvovirus in intestinal fragments of newborn dogs with diarrhea [220]. This technique also allows studying the efficiency of the vaccines based on in situ produced, noninfectious rotavirus-like particles (RVLPs) [221].

2. Conclusion

The different transmission electron microscopy modalities promote a fast and accurate diagnosis of the different gastroenteric viral agents, allowing prophylactic measures of control and prevention in the creations to be promptly instituted, avoiding animal losses and disastrous economic losses, and collaborating with the National Porcine and Bovine Agribusiness.

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References

- [1] Martella V, Banyai K, Matthijnssens J, Buonavoglia C, Ciarlet M. Zoonotic aspects of rotaviruses. *Veterinary Microbiology*. 2010;**140**:246-255
- [2] Cho YI, Han JI, Wang C, Cooper V, Schwartz K, Engelken T, Yoon KY. Case-control study of microbiological etiology associated with calf diarrhea. *Veterinary Microbiology*. 2013;**166**:375-385
- [3] Zhou X, Wang YH, Souvik G, Tang WF, Pang BB, Liu MQ, Peng JS, Zhou DJ, Kobayashi N. Genomic characterization of G3P[6], G4P[6] and G4P[8] human rotaviruses from Wuhan, China: Evidence for interspecies transmission and reassortment events. *Infection, Genetics and Evolution*. 2015;**33**:55-71
- [4] Svensson CLK, Emanuelson U, Olsson SO. Morbidity in Swedish dairy calves from birth to 90 days of age and individual calf-level risk factors for infectious diseases. *Preventive Veterinary Medicine*. 2003;**58**(3/4):179-197
- [5] Dhama K, Chauhan RS, Maheendran M, Malik SVS. Rotavirus diarrhoea in bovines and other domestic animals. *Veterinary Research Communications*. 2009;**33**:1-23
- [6] Saif LJ, Fernandez FM. Group A rotavirus veterinary vaccines. *Journal of Infectious Diseases*. 1996;**174**(Suppl. 1):S98-S106
- [7] Conner ME, Darlington RW. Rotavirus infection in foals. *American Journal of Veterinary Research*. 1980;**41**:1699-1703
- [8] ICTV2016 International Committee on Taxonomy of Viruses. Available from: <https://talk.ictvonline.org/taxonomy/>

- [9] Matthijnssens J, Otto PH, Ciarlet M, Desselberger U, Van Ranst M, Johne R. VP6-sequence-based cutoff values as a criterion for rotavirus species demarcation. *Archives of Virology*. 2012;**157**:1177-1182
- [10] Chandler-Bostock R, Hancox LR, Payne H, Iturriza-Gomara M, Daly JM, Mellits KH. Diversity of group A rotavirus on a UK pig farm. *Veterinary Microbiology*. 2015;**180**: 205-211
- [11] Uhde FL, Kaufmann T, Sager H, Albini S, Zanoni R, Schelling E, Meylan M. Prevalence of four enteropathogens in the faeces of young diarrhoeic dairy calves in Switzerland. *The Veterinary Record* 2008;**163**:362-366.
- [12] Langoni H, Linhares AC, Avila FA, Da Silva AV, Elias AO. Contribution to the study of diarrhea etiology in neonate dairy calves in São Paulo state, Brazil. *Brazilian Journal of Veterinary Research and Animal Science*. 2004;**41**:313-319
- [13] Bartels CJM, Holzhauer M, Jorritsma R, Swart WAJM, Lam TJGM. Prevalence, prediction and risk factors of enteropathogens in normal and non-normal faeces of young Dutch dairy calves. *Preventive Veterinary Medicine*. 2010;**93**:162-169
- [14] Izzo MM, Kirkland PD, Mohler VL, Perkins NR, Gunn AA, House JK. Prevalence of major enteric pathogens in Australian dairy calves with diarrhea. *Australian Veterinary Journal*. 2011;**89**:167-173
- [15] Cho Y, Yoon K-J. An overview of calf diarrhea – infectious etiology, diagnosis, and intervention. *Journal of Veterinary Science* 2014;**15**(1):1-17.
- [16] AlMawly J, Grinberg A, Prattley D, Moffat J, French N. Prevalence of endemic enteropathogens of calves in New Zealand dairy farms. *New Zealand Veterinary Journal*. 2015;**63**:147-152
- [17] Fukai K, Saito T, Inoue K, Sato M. Molecular characterization of novel P[14],G8 bovine group A rotavirus, Sun9, isolated in Japan. *Virus Research*. 2004;**105**:101-106
- [18] Estes M, Greenberg HB. Rotaviruses. In: Knipe DM, Howley PM, Cohen JL, Griffin DE, Lamb RA, Martin MA, Roizman B, Racaniello VR, editors. *Fields Virology*. Philadelphia: Lippincott Williams and Wilkins; 2013. p. 2013
- [19] Hayashi-Miyamoto M, Murakami T, Minami-Fukuda F, Tsuchiaka S, Kishimoto M, Sano K, Naoi K, Asano K, Ichimaru T, Haga K, Omatsu T, Katayama Y, Oba M, Aoki H, Shirai J, Ishida M, Katayama K, Mizutani T, Nagai M. Diversity in VP3, NSP3, and NSP4 of rotavirus B detected from Japanese cattle. *Infection, Genetics and Evolution*. 2017;**49**:97-103
- [20] Barman P, Ghosh S, Das S, Varghese V, Chaudhuri S, Sarkar S, Krishnan T, Bhattacharya SK, Chakrabarti A, Kobayashi N, Naik TN. Sequencing and sequence analysis of VP7 and NSP5 genes reveal emergence of a new genotype of bovine group B rotaviruses in India. *Journal of Clinical Microbiology*. 2004;**42**:2816-2818
- [21] Chasey D, Davies P. Atypical rotaviruses in pigs and cattle. *The Veterinary Record*. 1984;**114**:16-17

- [22] Chang KO, Parawani AV, Smith D, Saif LJ. Detection of group B rotaviruses in fecal samples from diarrheic calves and adult cows and characterization of their VP7 genes. *Journal of Clinical Microbiology*. 1997;**35**:2107-2110
- [23] McNulty MS, Logan EF. Longitudinal survey of rotavirus infection in calves. *The Veterinary Record* 1983;**13**:33-35.
- [24] Mebus CA. Bovine and ovine rotavirus. In: Dinter Z, Morein B. *Virus Infections of Ruminants*. New York: Elsevier Science, 1990. pp. 239-244.
- [25] Kalkanov I, Dinev I, Aleksandrov M, Dimitrov K, Zarkov I. Morphological and etiological investigations in a rotaviral enteritis outbreak in calves. *Kafkas Universitesi Veteriner Fakultesi Dergisi*. 2016;**22**(2):309-313
- [26] Mohanty SB, Dutta SK. *Veterinary Virology*. Philadelphia: Lea & Febiger; 1981. 372 p
- [27] Athanassious R, Marsolais G, Assaf R, Dea S, Descoteaux J-P, Dulude S, Montpetit C. Detection of bovine coronavirus and type A rotavirus in neonatal calf diarrhea and winter dysentery of cattle in Quebec: Evaluation of three diagnostic methods. *The Canadian Veterinary Journal*. 1994;**35**:163-169
- [28] Schroeder ME, Bounpheng MA, Rodgers S, Baker RJ, Black W, Naikare H, Velayudhan B, Sneed L, Szonyi B, Clavijo A. Development and performance evaluation of calf diarrhea pathogen nucleic acid purification and detection workflow. *Journal of Veterinary Diagnostic Investigation*. 2012;**24**:945-953
- [29] Alkan F, Ozkul A, Oguzoglu TC, Timurkan MO, Caliskan E, Martella V, Burgu I. Distribution of G (VP7) and P (VP4) genotypes of group A rotaviruses from Turkish calves with diarrhea, 1997-2008. *Veterinary Microbiology*. 2010;**141**:231-237
- [30] Alfieri AA, Alfieri AF, Beuttemmóller EA, Brito BG, Médici KC. Aspectos epidemiológicos da rotavirose suína na região Sudoeste do Estado do Paraná, Brasil. *Semina Ciencias Agrarias*. 1999;**20**:5-11
- [31] Paho: Pan American Health Organization. Rotaviral gastroenteritis. In: *Zoonoses and communicable diseases common to man and animals: chlamydioses, rickettsioses and viroses*. 3rd ed. Washington, USA: Paho; 2001. p. 286-294
- [32] Kindler E, Trojnar E, Heckel G, Otto PH, Johne R. Analysis of rotavirus species diversity and evolution including the newly determined full-length genome sequences of rotavirus F and G. *Infection, Genetics and Evolution*. 2013;**14**:58-67
- [33] Molinari BLD, Lorenzetti E, Otonel RAA, Alfieri AF, Alfieri AA. Species H rotavirus detected in piglets with diarrhea, Brazil, 2012. *Emerging Infectious Disease*. 2014;**20**(6):1019-1022
- [34] Saif LJ. *Reoviridae*. In: Mac Lachlan NJ, Dobovi EJ, editors. *Fenner Veterinary Virology IV*. 4th ed. Oxford, UK: Elsevier; 2011. p. 276-290

- [35] Martella V, Ciarlet M, Bányai K, Lorusso E, Arista S, Lavazza A, Pezzotti G, Decaro N, Cavalli A, Lucente MS, Corrente M, Elia G, Camero M, Tempesta M, Buonavoglia C. Identification of group A porcine rotavirus strains bearing a novel VP4 (P) genotype in Italian swine herds. *Journal of Clinical Microbiology* 2007;**45**:577-580.
- [36] Amimo JO, Vlasova AN, Saif LJ. Detection and genetic diversity of porcine group A rotaviruses in historic (2004) and recent (2011 and 2012) swine fecal samples in Ohio: predominance of the G9P[13] genotype in nursing piglets. *Journal of Clinical Microbiology*. 2013;**51**:1142-1151
- [37] Amimo JO, Junga JO, Ogara WO, Vlasova AN, Njahira MN, Maina S, Okoth EA, Bishop RP, Saif LJ, Djikeng A. Detection and genetic characterization of porcine group A rotaviruses in asymptomatic pigs in smallholder farms in East Africa: predominance of P[8] genotype resembling human strains. *Veterinary Microbiology* 2015;**175**(2-4):195-210.
- [38] Anh PH, Carrique-Mas JJ, Cuong NV, ThiHoa NT, Anh NL, Duy DT, Hien VB, PVT M, Rabaa MA, Farrar J, Baker S, Bryant JE. The prevalence and genetic diversity of group A rotaviruses on pig farms in the Mekong Delta region of Vietnam. *Veterinary Microbiology*. 2014;**170**:258-265
- [39] Bostock RC, Hancox LR, Nawaz S, Watts O, Iturriza-Gomara M, Mellits KM. Genetic diversity of porcine group A rotavirus strains in the UK. *Veterinary Microbiology*. 2014;**173**:27-37
- [40] Monini M, Zaccaria G, Ianiro G, Lavazza A, Vaccari G, Ruggeri FM. Full-length genomic analysis of porcine rotavirus strains isolated from pigs with diarrhea in Northern Italy. *Infection, Genetics and Evolution*. 2014;**25**:4-13
- [41] Ruiz VLA, Bersano JG, Carvalho AF, Catroxo MHB, Chiebao DP, Gregori F, Miyashiro S, Nassar AFC, Oliveira TMFS, Ogata RA, Scarcelli EP, Tonietti PO. Case-control study of pathogens involved in piglet diarrhea. *BMC Research Notes* 2016;**9**(22):1-7.
- [42] Qiao X, Li G, Wang X, Li X, Liu M, Li Y. Recombinant porcine rotavirus VP4 and VP4-LTB expressed in *Lactobacillus casei* induced mucosal and systemic antibody responses in mice. *BMC Microbiology*. 2009;**9**(249):1-11
- [43] Alfieri AA, Alfieri AF, Matos ACD, Lorenzetti E, Lobato ZIP. *Reoviridae*. In: Flores E, editor. *Virologia Veterinária*. 2nd ed. Santa Maria: UFSM; 2012
- [44] Graham DY, Sackman JW, Estes MK. Pathogenesis of rotavirus induced diarrhea—preliminary studies in miniature swine piglet. *Digestive Diseases and Sciences*. 1984;**29**:1028-1035
- [45] Estes MK. Rotaviruses and their replication. In: Fields BN, Knipe DM, editors. *Fields Virology*. New York: Raven Press; 1990. p. 1329-1352
- [46] Terret LA, Saif LJ. Serial propagation of porcine group C rotavirus (para rotavirus) in primary porcine kidney cell culture. *Journal of Clinical Microbiology*. 1987;**25**(7):1316-1319

- [47] Kim Y, Chang K-O, Straw B, Saif LJ. Characterization of group C rotaviruses associated with diarrhea outbreaks in feeder pigs. *Journal of Clinical Microbiology*. 1999; **37**(5):1484-1488
- [48] Smitalova R, Rodak L, Psikal I, Smid B. Isolation, immunochemical demonstration of field strains of porcine group A rotaviruses and electrophoretic analysis of RNA segments of group A and C rotaviruses. *Veterinary Medicine*. 2006;**51**(5):288-295
- [49] Dewey C, Carman S, Pasma T, Josephson G, McEwen B. Relationship between group A porcine rotavirus and management practices in swine herds in Ontario. *The Canadian Veterinary Journal*. 2003;**44**:649-653
- [50] Parreno V, Mino SL, Garaicoechea L, Barrandeguy M. Equine rotavirus in Argentinean foals: An overview. 10th IEIDC Abstracts. *Journal of Equine Veterinary Science*. 2016;**39**: S23-S25
- [51] Papp H, Lászlá B, Jakab F, Ganesh B, De Grazia S, Matthijnssens J, Ciarlet M, Martella V, Bányai K. Review of group A rotavirus strains reported in swine and cattle. *Veterinary Microbiology*. 2013;**165**:190-199
- [52] Stoneham SJ. Practical aspects of diarrhea in the foal with particular reference to rotavirus and gastroduodenal ulceration. *Equine Veterinary Education*. 1996;**8**(2):84-90
- [53] Ghosh S, Urushibara N, Chawla-Sarkar M, Krishnan T, Kobayashi N. Whole genomic analyses of asymptomatic human G1P[6], G2P[6] and G3P[6] rotavirus strains reveal inter genogroup reassortment events and genome segments of artiodactyl origin. *Infection, Genetics and Evolution*. 2013;**16**:165-173
- [54] Imagawa H, Kato T, Tsunemitsu H. Field study of inactivated equine rotavirus vaccine. *Japanese Society of Equine Science*. 2005;**16**:35-44
- [55] Cohen ND, Chaffin MK. Causes of diarrhea and enteritis in foals. *Compendium on Continuing Education for the Practising Veterinarian*. 1995;**17**(4):568-574
- [56] Fenger CK. Doenças dos potros. In: Reed SM, Bayly WM, editors. *Medicina interna eqüina*. Rio de Janeiro: Guanabara Koogan; 2000. p. 803-839
- [57] Payment P, Morin E. Minimal infective dose of the OSU strain of porcine rotavirus. *Archives of Virology*. 1990;**112**:277-282
- [58] Slovis NM, Elam J, Estrada M, Thao MF, Leutenegger CM. Comprehensive analysis of infectious agents associated with diarrhea in foals in Central Kentucky. In: *Proceedings of the 56th Annual Convention of the American Association of Equine Practitioners*. Baltimore, USA; 2010. p. 262
- [59] Lester G. Neonatologia. In: Savage CJ, editor. *Segredos em medicina de equinos*. Porto Alegre: Art Med; 2001. p. 247-264
- [60] Tillotson K, Traub-Dargatz JL. Gastrointestinal protectants and cathartics. *Veterinary Clinics of North America. Equine Practice*. 2003;**1993**:599-615

- [61] Conner ME, Gillespie JH, Schiff EI, Frey MS. Detection of rotavirus in horses with and without diarrhea by electron microscopy and rotazymetest. *The Cornell Veterinarian*. 1983;**73**(3):280-287
- [62] Batt RM, Embaye H, Van de Waal S, Burgess D, Edwards GB, Hart CA. Application of organ culture of small intestine to the investigation of enterocyte damage by equine rotavirus. *Journal of Pediatric Gastroenterology and Nutrition* 1995;**20**(3):326-332.
- [63] Ntafis V, Fragkiadaki E, Xylouri E, Omirou A, Lavazza A, Martella V. Rotavirus-associated diarrhoea in foals in Greece. *Veterinary Microbiology*. 2010;**144**(3-4):461-465
- [64] Bailey KE, Gilkerson JR, Brownng GF. Equine rotaviruses-current understanding and continuing challenges. *Veterinary Microbiology*. 2013;**167**(1-2):135-144
- [65] Fitzgerald TA, Munoz M, Wood AR, Snodgrass DR. Serological and genomic characterisation of group A rotaviruses from lambs. *Archives of Virology*. 1995;**140**:1541-1548
- [66] Munoz M, Alvarez M, Lanza I, Carmenes P. An outbreak of diarrhea associated with atypical rotaviruses in goat kids. *Research in Veterinary Science* 1995;**59**:180-182.
- [67] Snodgrass DR, Herring JA, Gray EW. Experimental rotavirus infection in lambs. *Journal of Comparative Pathology*. 1976;**86**:637-642
- [68] Alkan F, Gulyaz V, Timurkan OM, Iyisan S, Ozdemir S, Turan N, Buonavoglia C, Martella V. A large outbreak of enteritis in goat flocks in Marmara, Turkey, by G8P [1] group A rotaviruses. *Archives of Virology*. 2012;**157**:1183-1187
- [69] Gazal S, Taku AK, Kumar B. Predominance of rotavirus genotype G6P [11] in diarrheic lambs. *Veterinary Journal*. 2012;**193**:299-300
- [70] Papp H, Malik Y, Farkas S, Jakab F, Martella V, Banyai K. Rotavirus strains in neglected animal species, including lambs, goats and camelids. *Virus Diseases*. 2014;**25**:215-222
- [71] Theil KW, Lance SE, McCloskey CM. Rotaviruses associated with neonatal lamb diarrhea in two Wyoming shed-lambing operations. *Journal of Veterinary Diagnostic Investigation*. 1996;**8**:245-248
- [72] Luchs A, Timenetsky MCST. Gastroenterite por rotavírus do grupo A: era pós-vacinal, genótipos e transmissão zoonótica. *Einstein*. 2016;**14**(2):278-287.
- [73] Buzinaro MG, Samara SI, Carvalho AAB, Pontes JV, Salles R, Silva DG. Detecção e isolamento de rotavírus caprino do grupo A. *Arquivos do Instituto Biológico*. 2011;**78**(2):301-304
- [74] Munoz M, Alvarez M, Lanza I, Carmenes P. Role of enteric pathogens in the aetiology of neonatal diarrhoea in lambs and goat kids in Spain. *Epidemiology and Infection*. 1996;**117**:203-211
- [75] Snodgrass DR, Wells PW. The immunoprophylaxis of rotavirus infections in lambs. *The Veterinary Record*. 1978;**102**:146-148

- [76] Wells PW, Snodgrass DR, Herring JA, Dawson AM. Antibody titres to lamb rotavirus in colostrum and milk of vaccinates ewes. *The Veterinary Record*. 1978;**103**:46-48
- [77] Woo PCY, Lau SKP, Huang Y, Yuen K-Y. Coronavirus diversity phylogeny and interspecies jumping. *Experimental Biology and Medicine*. (Maywood). 2009;**234**(10):1117-1127
- [78] Kapil S, Lamm CG, McVey DS, et al. Detection of bovine respiratory coronavirus in beef cattle In: Proceedings of the 27th annual meeting of the American Society of Virologists. Cornell University, Ithaca, NY, July 15, 2008. p. 9-10
- [79] Boileau MJ, Kapil S. Bovine coronavirus associated syndromes. *Veterinary Clinics of North America: Food Animal Practice*. 2010;**26**:123-146
- [80] Vlasova AN, Saif LJ. Biological aspects of the interspecies transmission of selected coronaviruses. In: Singh SK, editor. *Viral infections and global change*. First ed. Hoboken, NJ: Wiley Blackwell Press; 2014. pp. 393-418
- [81] Holmes KV, Lai MMC. *Coronaviridae: The viruses and their replication*. In: Fields BN, Knipe DM, Howley PM, editors. *Virology*. 3rd ed. Philadelphia: Lippincott-Raven Publishers; 1996. p. 1075-1093
- [82] Masters PS. The molecular biology of coronaviruses. *Advances in Virus Research*. 2006; **66**:193-292
- [83] Cleri DJ, Ricketti AJ, Vernaleo JR. Severe acute respiratory syndrome (SARS). *Infectious Disease Clinics of North America*. 2010;**24**:175-202
- [84] Evermann JF, Benfield DA. Coronaviral infections. In: Williams ES, Barber IK, editors. *Infectious Diseases of Wild Mammals*. 3rd ed. Ames (IA): Iowa State University Press; 2001. p. 245-253
- [85] Gunn AA, Naylor JA, House JK. Diarrhea. In: Smith BP, editor. *Large Animal Internal Medicine*. 4th ed. St. Louis (MO): Mosby Elsevier; 2009. p. 340-363
- [86] Kapil S, Trent AM, Goyal SM. Excretion and persistence of bovine coronavirus in neonatal calves. *Archives of Virology*. 1990;**115**(1-2):127-132
- [87] Tsunemitsu H, El-Kanawati ZR, Smith DR, Reed HH, Saif LJ. Isolation of coronaviruses antigenically indistinguishable from bovine coronavirus from wild ruminants with diarrhea. *Journal of Clinical Microbiology*. 1995;**33**(12):3264-3269
- [88] White ME, Schukken Hein Y, Tanksley B. Space-time clustering of, and risk factors for, farmer-diagnosed winter dysentery in dairy cattle. *The Canadian Veterinary Journal*. 1989;**30**(12):948-951
- [89] Jactel B, Espinasse J, Viso M, Valiergue H. An epidemiological study of winter dysentery in fifteen herds in France. *Veterinary Research Communications* 1990; **14**(5):367-379.
- [90] Abuelo A, Perez-Santos MA. Winter dysentery (coronavirus infection) outbreak in a dairy herd in Galicia (northwestern Spain). *The Veterinary Record*. 2016;**4**:e000328

- [91] Durham PJK, Hassard LE, Norman GR, Yemen RL. Viruses and virus-like particles detected during examination of feces from calves and piglets with diarrhea. *The Canadian Veterinary Journal*. 1989;**30**:876-881
- [92] Decaro N, Mari V, Desario C, Campolo M, Elia G, Martella V, Greco G, Cirone F, Colaianne ML, Cordioli P, Buonavoglia C. Severe outbreak of bovine coronavirus infection in dairy cattle during the warmer season. *Veterinary Microbiology*. 2008;**126**:30-39
- [93] Takahashi E, Akashi H, Inaba Y. Bovine epizootic diarrhea resembling winter dysentery caused by bovine coronavirus. *Japan Agricultural Research Quarterly*. 1983;**17**(3): 185-190
- [94] Takiuchi E, Barry AF, Alfieri AF, Filippsen P, Alfieri AA. An outbreak of winter dysentery caused by bovine coronavirus in a high-production dairy cattle herd from a tropical country. *Brazilian Archives of Biology and Technology*. 2009;**52**(Special):45-49
- [95] Cho KO, Halbur PG, Bruna JD, Sorden SD, Yoon KJ, Janke BH, Chang KO, Saif LJ. Detection and isolation of coronavirus from feces of three herds of feedlot cattle during outbreaks of winter dysentery-like disease. *Journal of the American Veterinary Medical Association*. 2000;**217**:1191-1194
- [96] Saif LI, Redman DR, Brock KV, Kohler EM, Heckert RA. Winter dysentery in adult dairy cattle: Detection of coronavirus in the faeces. *The Veterinary Record*. 1988;**123**:300-301
- [97] Traven M, Naslund K, Linde N, Linde B, Silván A, Fossum C, Hedlund KO, Larsson B. Experimental reproduction of winter dysentery in lactating cows using BCV-comparison with BCV infection in milk-fed calves. *Veterinary Microbiology*. 2001;**81**(2):127-151
- [98] Saif LJ, Brock KV, Redman DR, Kohler EM. Winter dysentery in dairy herds: Electron microscopic and serological evidence for an association with coronavirus infection. *The Veterinary Record*. 1991;**128**(19):447-449
- [99] Heckert RA, Saif LJ, Myers GW. Development of protein-a gold immunoelectron microscopy for detection of bovine coronavirus in calves – comparison with Elisa and direct immunofluorescence of nasal epithelial-cells. *Veterinary Microbiology*. 1989;**19**: 217-231
- [100] Dea S, Garzon S. Identification of coronaviruses by the use of indirect protein-A immunogold electron microscopy. *Journal of Veterinary Diagnostic Investigation*. 1991;**3**:297-305
- [101] Saif LJ, Redman DR, Moorhead PD, Theil KW. Experimentally induced coronavirus infections in calves – Viral replication in the respiratory and intestinal tracts. *American Journal of Veterinary Research*. 1986;**47**:1426-1432
- [102] Soltana MA, Tsai Y-L, Lee P-YA, Tsai C-F, Chang H-FG, Wang H-TT, Wilkes RP. Comparison of electron microscopy, ELISA, real time RT-PCR and insulated isothermal RT-PCR for the detection of rotavirus group A (RVA) in feces of different animal species. *Journal of Virological Methods*. 2016;**235**:99-104

- [103] Cortese VS. Neonatal immunology. *The Veterinary Clinics of North America. Food Animal Practice*. 2009;**25**(1):221-227
- [104] Radostits OM, Blood DC, Gay CC. *Clínica Veterinária. Um tratado de doenças dos bovinos, ovinos, suínos, caprinos e eqüinos*. 9th ed. Guanabara Koogan: Rio de Janeiro; 2002. p. 1737
- [105] Saif LJ, Wesley RD. Transmissible gastroenteritis and porcine respiratory coronavirus. In: Straw BE, editor. *Diseases of Swine*. 8th ed. 1999. p. 295-325
- [106] Sestak K, Saif LJ. Porcine coronavirus. In: *Trends in Emerging Viral Infection of Swine*. Ames: Iowa State Press; 2002. p. 321-330
- [107] OIE. Manual of diagnostic tests & vaccines for terrestrial animals. In: *Transmissible Gastroenteritis*. 6th ed. Paris: França; 2008. p. 1153-1163
- [108] Martins AMCRPF, Bersano JG, Ogata R, Amante G, Nastari BDB, Catroxo MHB. Diagnosis to detect porcine transmissible gastroenteritis virus (TGEV) by optical and transmission electron microscopy techniques. *International Journal of Morphology*. 2013;**31**(2):706-715
- [109] Wyllie AH, Morris RG, Smith AL, Dunlop D. Chromatin cleavage in apoptosis: Association with condensed chromatin morphology and dependence on molecular synthesis. *The Journal of Pathology*. 1984;**142**:67-77
- [110] Sur J-H, Doster AR, Osorio FA. Apoptosis induced in vivo during acute infection by porcine reproductive and respiratory syndrome virus. *Veterinary Pathology*. 1998; **35**:506-514
- [111] Haelterman EO. On the pathogenesis of transmissible gastroenteritis of swine. *Journal of the American Veterinary Medical Association*. 1972;**160**:534-540
- [112] Kemeny LJ, Wiltsey VL, Riley JL. Upper respiratory infection of lactating sows with transmissible gastroenteritis virus following contact exposure to infected piglets. *The Cornell Veterinarian*. 1975;**65**:352-362
- [113] Saif LJ, Sestak K. Transmissible gastroenteritis virus and porcine respiratory coronavirus. In: Straw BE et al., editors. *Diseases of Swine*. 9th ed. Ames, Iowa: Blackwell Publishing; 2006. p. 489-516
- [114] Geering WA, Forman AJ, Nunn MJ. *Exotic Diseases of Animals: A Field Guide for Australian Veterinarians*. Canberra: Australian Government Publishing; 1995. p. 252-258
- [115] Risco C, Anton IM, Suné C, Pedregosa AM, Martin-Alonso JM, Parra F, Carrascosa JL, Enjuanes L. Membrane protein molecules of transmissible gastroenteritis coronavirus also expose the carboxy-terminal region on the external surface of the virion. *Journal of Virology*. 1995;**69**(9):5269-5277
- [116] Cappellaro CEMPDM, Bersano JG, Catroxo MHB, Mueller SBK. Presença de coronavírus associado a surto de gastroenterite em suínos no município de Mairinque, SP, no ano de 1988. *Arquivos do Instituto Biológico*. 1998;**65**(2):111-116

- [117] Bersano JG, Castro AMMG, Brandão PE, Villalobos EMC, Cortez A, Soares RM, Ruiz VLA, Leomil H, Catroxo MHB, Monteiro RM, Ogata RA, Richtzenhain LJ. Detecção mista por circovírus suíno (PCV) e coronavírus em suíno: Relato de caso. *Arquivos do Instituto Biológico*. 2003;**70**(Suppl. 3):75
- [118] Barrera-Vale M, Landa HAD, Beiras AMA, Portal AC, Batista ER, Redondo AV, Varela RU, Lepoureau MTF. Transmissible gastroenteritis in Cuba: Experimental reproduction of the disease and molecular characterization of the virus. *Spanish Journal of Agricultural Research* 2005;**3**(3):267-274.
- [119] Nogales A, Márquez-Jurado S, Galán C, Enjuanes L, Almazán F. Transmissible gastroenteritis coronavirus RNA-dependent RNA polymerase and nonstructural proteins 2, 3, and 8 are incorporated into viral particles. *Journal of Virology*. 2012;**86**(2):1261-1266
- [120] Sobestiansky J, Barcellos DESN, Morés N, Oliveira SJ, Carvalho LFOS, Moreno AM, Roehe PM. *Clínica e patologia suína*. Goiânia: Art 3 Impressos Especiais; 1999. p. 402
- [121] Pospischil A, Stuedli A, Kiupel M. Diagnostic notes update on porcine epidemic diarrhoea. *Journal of Swine Health and Production*. 2002;**10**:81-85
- [122] Wood EN. An apparently new syndrome of porcine epidemic diarrhoea. *The Veterinary Record*. 1977;**100**:243-244
- [123] Cui X. Studies on the detection of porcine epidemic diarrhea virus by immunofluorescent techniques. *Chinese Journal of Preventive Veterinary Medicine*. 1990;**5**:20-24
- [124] Fan H, Zhang J, Ye Y, Tong T, Xie K, Liao M. Complete genome sequence of a novel porcine epidemic diarrhea virus in south China. *Journal of Virology*. 2012;**86**:10248-10249
- [125] Son D, Park B. Porcine epidemic diarrhea virus: A comprehensive review of molecular epidemiology, diagnosis, and vaccines. *Virus Genes*. 2012;**44**:167-175
- [126] Wang L, Byrum B, Zhang Y. New variant of porcine epidemic diarrhea virus, United States, 2014. *Emerging Infectious Diseases*. 2014;**20**:917-919
- [127] Quevedo-Valle MV. Porcine epidemic diarrhea outbreak in Peru. In: *International Conference on Swine Enteric Coronavirus Diseases*. Chicago, IL, USA; Sep 23-25, 2014
- [128] Piñeros R, Galvis JDM. Coronavirus en porcinos: importancia y presentación del virus de la diarrea epidêmica porcina (PEDV) en Colombia. *Revista de Medicina Veterinaria*. 2015;**29**:73-89
- [129] Harris HDL. Porcine Epidemic Diarrhea. *The Merck Manual*. Merck, Sharpe and Dohme Corp. Kenilworth, NJ, USA: Iowa State University; 2012
- [130] Dastjerdi A, Carr J, Ellis R, Steinbach F, Williamson S. Porcine epidemic diarrhea virus among farmed pigs. *Ukraine Emerging Infectious Diseases*. 2015;**21**:2235-2237
- [131] Lee C. Porcine epidemic diarrhea virus: An emerging and re-emerging epizootic swine virus. *Virology Journal*. 2015;**12**:193

- [132] Ducatelle R, Coussement W, Debouck P, Hoorens J. Pathology of experimental CV777coronavirus enteritis in piglets. II. Electron microscopic study. *Veterinary Pathology*. 1982;**19**:57-66
- [133] Saif LJ, Pensaert MD, Sestak K, Yeo SG, Jung K. Coronaviruses. In: Zimmerman JJ, Karriker LA, Ramirez A, Schwartz KJ, Stevenson CW, editors. *Diseases of Swine*. 10th ed. Hoboken, NJ: Wiley-Blackwell; 2012. pp. 1081-1091.
- [134] Kim Y, Lee C. Porcine epidemic diarrhea virus induces caspase-independent apoptosis through activation of mitochondrial apoptosis-inducing factor. *Virology*. 2014;**460-461**(1):180-193
- [135] Xu J, Guo H-C, Wei Y-Q, Dong H, Han S-C, Ao D, Sun D-H, Wang H-M, Cao S-Z, Sun S-Q. Self-assembly of virus-like particles of canine parvovirus capsid protein expressed from *Escherichia coli* and application as virus-like particle vaccine. *Applied Microbiology and Biotechnology*. 2014;**98**:3529-3538
- [136] Dhama K, Pawaiya RVS, Chakraborty S, Tiwari R, Saminathan M, Verma AK. Coronavirus infection in equines: A review. *Asian Journal of Animal and Veterinary Advances*. 2014;**9**(3):167-176
- [137] Balasuriya UBR. *Coronaviridae*. In: McVey DS, Kennedy M, Chengappa MM, editors. *Veterinary Microbiology*. 3rd ed. Ames, IA: Wiley-Blackwell; 2013. p. 456-473
- [138] Zhang J, Guy JS, Snijder EJ, Denniston DA, Timoney P, Balasuriya UB. Genomic characterization of equine coronavirus. *Virology*. 2007;**369**:92-104
- [139] Guy JS, Breslin JJ, Breuhaus B, Vivrette S, Smith LG. Characterization of a coronavirus isolated from a diarrheic foal. *Journal of Clinical Microbiology*. 2000;**38**:4523-4526
- [140] Oue Y, Morita Y, Kondo T, Nemoto M. Epidemic of equine coronavirus at Obihiro Race course, Hokkaido, Japan in 2012. *Journal of Veterinary Medical Science*. 2013;**75**:261-1265
- [141] Mischczak F, Tesson V, Kin N, Dina J, Balasuriya UB, Pronost S, Vabret A. First detection of equine coronavirus (ECoV) in Europe. *Veterinary Microbiology*. 2014;**171**:206-209
- [142] Pusterla N, Holzenkaempfer N, Mapes S, Kass P. Prevalence of equine coronavirus in nasal secretions from horses with fever and upper respiratory tract infection. *The Veterinary Record*. 2015;**177**:289
- [143] Kooijman LJ, Mapes SM, Pusterla N. Development of an equine coronavirus-specific enzyme-linked immunosorbent assay to determine serologic responses in naturally infected horses. *Journal of Veterinary Diagnostic Investigation*. 2016;**28**:414-418
- [144] Cappellaro CEMPDM, Catroxo MHB, Suga O, Mueller SBK, Oliveira JM, Carrascosa AATA, Scatolini CM. Presença de coronavírus em surto de enterite em eqüinos. *O Biológico*. 1988;**54**(7/12):49-51
- [145] Meirelles MG, Araújo LL, Friedrich Junior F, Flores EF, Nogueira CEW. Enterite associada à infecção por coronavírus em potros puro sangue inglês em um haras no Rio Grande do Sul. *Arquivos do Instituto Biológico*. 2011;**78**(4):605-608

- [146] Oue Y, Ishihara R, Edamatsu H, Morita Y, Yoshida M, Yoshima M, Hatama S, Murakami K, Kanno T. Isolation of an equine coronavirus from adult horses with pyrogenic and enteric disease and its antigenic and genomic characterization in comparison with the NC99 strain. *Veterinary Microbiology*. 2011;**150**:41-48
- [147] Pusterla N, Mapes S, Wademan C, White A, Ball R, Sapp K, Burns P, Ormond C, Butterworth K, Bartol J, Magdesian KG. Emerging outbreaks associated with equine coronavirus in adult horses. *Veterinary Microbiology*. 2013;**162**:228-231
- [148] Imagawa H, Fukunaga Y, Kamada M. Detection of neutralizing antibody against calf diarrheal coronavirus in horse serum. *Bulletin of Equine Research Institute*. 1990;**27**:25-30
- [149] Anzai T, Fukunaga Y, Matsumura T, Imagawa H, Oikawa M. Serological examination for viral infection among young racehorses transported by vehicle over a long distance. *Journal of Equine Science*. 2001;**12**:135-137
- [150] Hemida MG, Chu DK, Perera RA, Ko RL, So RT, Ng BC, Chan SM, Chu S, Alnaeem AA, Alhammadi MA, Webby RJ, Poon LL, Balasuriya UB, Peiris M. Coronavirus infections in horses in Saudi Arabia and Oman. *Transboundary and Emerging Diseases*. 2017:1-11
- [151] Daves E, Rush BR, Cox J. Neonatal enterocolitis associated with coronavirus infection in a foal: A case report. *Journal of Veterinary Diagnostic Investigation*. 2000;**12**:153-156
- [152] Ward ACS, Evermann JF, Reed SM. Presence of coronavirus in diarrheic foals. *Veterinary Medicine, Small Animal Clinician*. 1983;**78**:563-565
- [153] Suzuki K, Matsui Y, Miuraa Y, Sentsui H. Equine coronavirus induces apoptosis in cultured cells. *Veterinary Microbiology*. 2008;**129**:390-395
- [154] Traub-Dargatz JL, Gay CC, Evermann JF, Ward AC, Zeglen ME, Gallina AM, Salman MD. Epidemiologic survey of diarrhea in foals. *Journal of the American Veterinary Medical Association*. 1988;**192**:1553-1556
- [155] Dwyer RM, Powell DG, Roberts W, Donahue M, Lyons ET, Osborne M, Woode GA. study of the etiology and control of infectious diarrhea among foals in central Kentucky. *Proceedings of the American Association of Equine Practitioners*. 1990;**36**:337-355
- [156] Mair TS, Taylor FGR, Harbour DA, Pearson GR. Concurrent cryptosporidium and coronavirus infections in an Arabian foal with combined immunodeficiency syndrome. *The Veterinary Record*. 1990;**126**:127-130
- [157] Browning GF, Chalmers RM, Snodgrass DR, Batt RM, Hart CA, Ormarod SE, Leadon D, Stoneham SJ, Rosedale PD. The prevalence of enteric pathogens in diarrhoeic thoroughbred foals in Britain and Ireland. *Equine Veterinary Journal*. 1991;**23**(6):405-409
- [158] Pusterla N, Vin R, Leutenegger C, Mittel LD, Divers TJ. Equine coronavirus: An emerging enteric virus of adult horses. *Equine Veterinary Education*. 2016;**28**(4):216-223
- [159] CNA, 2016. Available from: <http://www.cnabrazil.org.br/noticias/brasil-pode-se-tornar-o-maior-produtor-de-carne-bovina-do-mundo>.

- [160] Bauermann FV, Harmon A, Flores EF, Falkenberg SM, Reecy JM, Ridpath JF. In vitro neutralization of HoBi-like viruses by antibodies in serum of cattle immunized with inactivated or modified live vaccines of bovine viral diarrhea viruses 1 and 2. *Veterinary Microbiology*. 2013;**166**(1-2):242-245
- [161] Bazzucchi M, Bertolotti L, Giammarioli M, Rossi E, Petrini S, Rosati S, De Miaa GM. Complete genome sequence of a bovine viral diarrhea virus subgenotype 1g strain isolated in Italy. *Genome Announcements*. 2017;**5**(7):1-2
- [162] Evans CA, Lanyon SR, Reichel MP. Investigation of AGID and two commercial ELISAs for the detection of bovine viral diarrhea virus-specific antibodies in sheep serum. *Journal of Veterinary Diagnostic Investigation*. 2017;**29**(2):181-185
- [163] Darweesh MF, Rajput MKS, Braun LJ, Ridpath JF, Neill JD, Chase C. Characterization of the cytopathic BVDV strains isolated from 13 mucosal disease cases arising in a cattle herd. *Virus Research*. 2015;**195**:141-147
- [164] Gao S, Du J, Tian Z, Xing S, Luo J, Liu G, Chang H, Yin H. Genome sequence of a subgenotype 1a bovine viral diarrhea virus in China. *Genome Announcements*. 2016;**4**(6):1-2
- [165] Sato A, Tateishi K, Shinohara M, Naoi Y, Shiokawa M, Aoki H, Omori K, Mizutani T, Shirai J, Nagai M. Complete genome sequencing of bovine viral diarrhea Virus 1, subgenotypes 1n and 1o. *Genome Announcements*. 2016;**4**(1):e01744-15
- [166] Rêgo MJP, Batista Filho AFB, Oliveira PRF, Borges JM, França CAB, Ribeiro CP, Pituco EM, Pinheiro Junior JW. Epidemiological analysis of infection by the bovine viral diarrhea virus on family farms in Brazil. *Ciências Agrárias*. 2016;**37**(6):4119-4130
- [167] Brito WMED, Souza WJ, Vieira S, Linhares DCL, Barbosa ACVC, Alfaia BT. Serological study on bovine viral diarrhea in nonvaccinated dairy herds with reproductive disorders from Goiás. *Virus Reviews & Research*. 2002;**7**(1):144
- [168] Chaves NP, Bezerra DC, Sousa VE, Santos HP, Pereira HM. Frequência de anticorpos e fatores de risco para a infecção pelo vírus da diarréia viral bovina em fêmeas bovinas leiteiras não vacinadas na região amazônica maranhense, Brasil. *Ciência Rural*. 2010;**40**:1448-1451
- [169] Figueiredo HCP, Vieira PR, Lage AP, Leite RC. Prevalência de anticorpos contra o vírus da Diarréia Viral Bovina a vírus em Minas Gerais, Brasil. *Revista Brasileira de Reprodução Animal*. 1997;**121**:11-15
- [170] Quincozes CG, Fischer G, Hübner SO, Vargas GD, Vidor T, Brod CS. Prevalência e fatores associados à infecção pelo vírus da diarréia viral bovina na região Sul do Rio Grande do Sul. *Semina: Ciências Agrárias*. 2007;**28**:269-276
- [171] Simmonds P, et al. Family *Flaviviridae*. In: King AMQ et al., editors. *Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses*. San Diego, CA: Elsevier Academic; 2011. pp. 1003-1020.

- [172] Goens D. The evolution of bovine viral diarrhea: A review. *The Canadian Veterinary Journal*. 2002;**43**:946-954
- [173] Vilcek S, Paton DJ, Durkovic B, Strojny L, Ibata G, Moussa A, Loitsch A, Rossmanith W, Vega S, Scicluna MT, Paifi V. Bovine viral diarrhoea virus genotype 1 can be separated into at least eleven genetic groups. *Archives of Virology*. 2001;**146**:99-115
- [174] Ridpath JF, Fulton RW, Kirkland PD, Neill JD. Prevalence and antigenic differences observed between bovine viral diarrhea virus subgenotypes isolated from cattle in Australia and feedlots in the southwestern United States. *Journal of Veterinary Diagnostic Investigation*. 2010;**22**:184-191
- [175] Bazzucchi M, Bertolotti L, Giammarioli M, Casciari C, Rossi E, Rosati S, De Mia GMM. Complete genome sequence of a bovine viral diarrhea virus subgenotype 1 h strain isolated in Italy. *Genome Announcements*. 2016;**5**(8):1-2
- [176] Mósená AC, Weber MN, Cibulski SP, Silveira S, Silva MS, Mayer FQ, Canal CW. Genomic characterization of a bovine viral diarrhea virus subtype 1i in Brazil. *Archives of Virology*. 1624;**2017**:1119-1123
- [177] Peterhans E, Bachofen C, Stalder H, Schweizer M. Cytopathic bovine viral diarrhea viruses (BVDV): Emerging pestiviruses doomed to extinction. *Veterinary Research*. 2010;**41**(6):1-14
- [178] Brownlie J. The pathogenesis of bovine viral diarrhea virus infections. *Revue Scientifique Et Technique OIE*. 1990;**9**:43-59
- [179] Baker JC. The clinical manifestations of bovine viral diarrhea infection. *The Veterinary Clinics of North America*. 1995;**11**(3):425-445
- [180] USDA – United States Department of Agriculture – Animal and Plant Health Inspection Service. Bovine viral diarrhea (BVD) management practices and detection in bulk tank milk in the United States, 2007. Fort Collins, USA: USDA-APHIS-VS-CEAH, 2008
- [181] Radostits OM, Gay CC, Hinchcliff KW. *Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs, and Goats*. 10th ed. Edinburgh: Saunders-Elsevier; 2007. 2156 p
- [182] Flores EF. Diagnóstico laboratorial das infecções víricas. In: Flores, EF (Org). *Virologia Veterinária*. Santa Maria: UFSM, 2007. pp. 295-326
- [183] Ohmann HB. Electron microscopy of bovine virus diarrhea virus. *Revue Scientifique et Technique OIE*. 1990;**9**:61-73
- [184] Catroxo MHB, AMCRPF M, Petrella S, Pongiluppi T, Lopes D. Identification of flavivirus in large intestine of cattle by transmission electron microscopy using negative staining (rapid preparation) and immunolabelling with colloidal gold particles techniques. In: 21st Congress of the Brazilian Society of Microscopy and Microanalysis (XXI CSBMM). Búzios, RJ, Brazil; August 26-29, 2007

- [185] Gao Y, Wang S, Du R, Wang Q, Sun C, Wang N, Zhang P, Zhang L. Isolation and identification of a bovine viral diarrhea virus from sika deer in china. *Virology Journal*. 2011;**8**(83):1-6
- [186] Liu H, Li Y, Gao M, Wen K, Jia Y, Liu X, Zhang W, Ma B, Wang J. Complete genome sequence of a bovine viral diarrhea virus 2 from commercial fetal bovine serum. *Journal of Virology*. 2012;**86**(18):1023
- [187] Wegelt A, Reimann I, Granzow H, Beer M. Characterization and purification of recombinant bovine viral diarrhea virus particles with epitope-tagged envelope proteins. *The Journal of General Virology*. 2011;**92**(Pt 6):1352-1357
- [188] Rajukumar K, Karikalan M, Kumar M, Mishra N, Behera SP, Kulkarni DD. Comparison of immune electron microscopy with direct negative staining for detection of bovine viral diarrhoea virus. *Indian Journal of Veterinary Pathology*. 2015;**39**(3):235-238
- [189] Weiskircher E, Aligo J, Ning G, Konan KV. Bovine viral diarrhea virus NS4B protein is an integral membrane protein associated with Golgi markers and rearranged host membranes. *Virology Journal*. 2009;**6**:185
- [190] Bauermann FV, Falkenberg SM, Vander BL, Decaro N, Brodersen BW, Harmon A, Hessman B, Flores EF, Ridpath JF. Generation of calves persistently infected with hoBi-like pestivirus and comparison of methods for detection of these persistent infections. *Journal of Clinical Microbiology*. 2014;**52**(11):3845-3852
- [191] Schramlova J, Arientova SH, D. The role of electron microscopy in the rapid diagnosis of viral infections – review. *Folia Microbiologica*. 2010;**55**(1):88-101
- [192] Curry A, Appleton H, Dowsett B. Application of transmission electron microscopy to the clinical study of viral and bacterial infections: present and future. *Micron*. 2006;**37**:91-106
- [193] Hazelton PR, Gelderblom HR. Electron microscopy for rapid diagnosis of infectious agents in emergent situations. *Emerging Infectious Diseases*. 2003;**9**:294-303
- [194] Brenner S, Horne RW. A negative staining method for high-resolution electron microscopy of viruses. *Biochem Biophys Acta*. 1959;**34**:103
- [195] Palmer EL, Martin ML. *Electron Microscopy in Viral Diagnosis*. 2nd ed. Boca Raton, FL: CRC; 2000. 194 p
- [196] Harris KM, Perry E, Bourne J, Feinberg M, Ostroff L, Hurlburt J. Uniform serial sectioning for transmission electron microscopy. *The Journal of Neuroscience*. 2006;**26**:12101-12103
- [197] Mast J, Demeestere L. Electron tomography of negatively stained complex viruses: application in their diagnosis. *Diagnostic Pathology*. 2009;**4**(5):1-7
- [198] Roingeard P. Viral detection by electron microscopy: Past, present and future. *Biology of the Cell*. 2008;**100**(8):491-501

- [199] Derrick KS. Quantitative assay for plant viruses using serologically specific electron microscopy. *Virology*. 1973;**56**:652-653
- [200] Gerna G, Sarasini A, Passarini N, Torsellini M, Parea M, Battaglia M. Comparative evaluation of a commercial enzyme-linked immunoassay and solid-phase immune electron microscopy for rotavirus in stool specimens. *Journal of Clinical Microbiology*. 1987;**25**:1137-1139
- [201] Humphrey CD, Cook Jr EH, Bradley DW. Identification of enterically transmitted virus particles by solid phase immune electron microscopy. *Journal of Virological Methods* 1990; **29**:177-188.
- [202] Lewis DC. Three serotypes of Norwalk-like virus demonstrated by solid phase immune electron microscopy. *Journal of Medical Virology*. 1990;**30**:77-81
- [203] Lavazza A, Tittarelli C, Cerioli M. The Use of convalescent sera in immune-electron microscopy to detect non-suspected/new viral agents. *Viruses*. 2015;**7**:2683-2703
- [204] Katz D, Kohn A. Immunosorbent electron microscopy for detection of viruses. *Advances in Virus Research*. 1984;**29**:169-194
- [205] Fields BN, Knipe DM, Howley PM, editors. *Fields virology*. 3rd ed. Philadelphia: Lippincott-Raven; 1996
- [206] Doane FW, Anderson M. *Electron Microscopy in Diagnostic Virology – A Practical Guide and Atlas*. Cambridge: Cambridge University Press; 1987 178p
- [207] Anderson N, Doane FW. Specific identification of enteroviruses by immuno-electron microscopy using a serum-in-agar diffusion method. *Canadian Journal of Microbiology*. 1973;**19**:585-589
- [208] Milne RG, Luisoni E. Rapid high-resolution immune electron microscopy of plant viruses. *Virology*. 1975;**68**:270-274
- [209] Kjeldsberg E. Use of gold IgG complexes and human antisera for electron microscopy identification of hepatitis A virus and polioviruses. *Journal of Virological Methods*. 1986;**13**:207
- [210] Patterson S, Oxford JS. Analysis of antigenic determinants on internal and external proteins of influenza virus and identification of antigenic subpopulations of virion in recent field isolates using monoclonal antibodies and immunogold labeling. *Archives of Virology*. 1986;**88**:189-202
- [211] Catroxo MHB, Gregori F, Brandão PE, Melo NA. Detection of rotavirus and coronavirus in calves during outbreak of diarrhea in Dourados, state of Mato Grosso do Sul, Brazil. *Biológico*. 2007;**69**(2):114
- [212] De Paul AL, Mukdsi JH, Petiti JP, Gutiérrez S, Quintar AA, Maldonado CA, Alicia I, Torres AI. In: Dehghani H, editor. *Immunoelectron Microscopy: A Reliable Tool for the*

Analysis of Cellular Processes, Applications of Immunocytochemistry. Rijeka, Croatia: In-Tech; 2012. p. 65-96

- [213] Rosso F, Papale F, Barbarisi A. Environmental scanning electron microscopy gold immunolabeling in cell biology. *Methods in Molecular Biology* (Clifton, N.J.). 2013; **931**:517-523
- [214] Hyatt AD, Eaton B. *Immuno-Gold Electron Microscopy in Virus Diagnosis and Research*. Boca Raton, Florida, USA: CRC Press; 2000. 448 p
- [215] Cheville NF, Stasko J. Techniques in electron microscopy of animal tissue. *Veterinary Pathology*. 2013;**10**:1-14
- [216] Payne HR, Storz J, Henk WG. Bovine coronavirus antigen in the host cell plasmalemma. *Experimental and Molecular Pathology*. 1990;**53**(2):152-159
- [217] Salanueva IJ, Carrascosa JL, Risco C. Structural maturation of the transmissible gastroenteritis coronavirus. *Journal of Virology*. 1999;**73**(10):7952-7964
- [218] Glauert AM, Lewis PR. *An Introduction to Fixation and Embedding Procedures and Their Safe Use in the Laboratory. Biological Specimen Preparation for Transmission Electron Microscopy*. London: Portland Press Ltd; 1998. 319 p
- [219] Fong CKY. Electron microscopy for the rapid detection and identification of viruses from clinical specimens. *The Yale Journal of Biology and Medicine*. 1989;**62**:115-130
- [220] Catroxo MHB, Martins AMCRPF. Veterinary diagnostic using Transmission Electron Microscopy. In: Khan M, editor. *The Transmission Electron Microscope*. Chap. 15. Rijeka, Croatia: In-Tech; 2015. p. 326-350
- [221] Meier AF, Suter M, Schrane EM, Humbel BM, Tobler K, Ackermann M, Andrea S, Laimbacher AS. Transfer of anti-rotavirus antibodies during pregnancy and in milk following maternal vaccination with a herpes simplex virus type-1 amplicon vector. *International Journal of Molecular Sciences*. 2017;**18**(431):1-26