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Review on Viral Causes of Calf Diarrhea

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Abstract: Calf diarrhea is a major economic burden to the bovine industry because of high mortality and morbidity worldwide. The major viral agents that have been implicated in calf diarrhea are: Bovine rotavirus (BRV), Bovine coronavirus (BCV) group A and Bovine viral diarrhea virus (BVD) 1 and 2 as viral agents and occasionally caused by some other viruses such as, Bovine Togovirus (BTV), Bovine Norovirus (BNoV) and Bovine Nebovirus (BNeV). Infection with the viruses reduces the absorptive capacity of the intestines because of destruction of enterocytes and disrupts reabsorption of water, thereby leading to loss of fluid and electrolytes; as a result of the calf may sick or die. Diarrhea may also cause a vital public health problem due to its potential zoonotic importance and environmentally distribution. Rotaviruses and Coronavirus have a wide host range, infecting many animal species as well as humans. Various diagnostic tests such as ELISA, PCR, cell culture etc. are most commonly used for the rapid detection of various bacterial and viral pathogens in clinical specimens from diarrheic calves. There are also other non-infectious factors that can cause calf diarrhea, such as insufficient uptake of colostrum, poor sanitation, stress and cold weather, could cause neonatal calf diarrhea. There is no specific treatment for viral infections, but providing supportive care and management such as; providing adequate colostrum, keeping good hygiene and sanitation practices would help to reduce calf morbidity and mortality in dairy farms. Using vaccines, biosecurity measures; such as isolating sick animals, cleaning and disinfecting premises, improving good management system are also some important points to reduce the disease burden.

Key words: Calf Diarrhea · Colostrum · Coronavirus · Pathogenesis · Rotavirus · Transmission

INTRODUCTION

Calf diarrhea (also known as calf scouring) is a commonly reported disease and a major cause of economic loss to cattle producers. The 2007 National Animal Health Monitoring System (NAHMS) for U.S. dairy reported that 57% of weaning calf mortality was due to diarrhea and most cases occurred in calves less than one month old. A similar mortality rate (53.4%) for dairy calves due to calf diarrhea was recently reported in Korea [1].

Calf diarrhea that cause morbidity and mortality is the results of the complex interaction of the management practices, the environment, infectious agents and the animal itself. Mortality of neonates was mainly attributed to conditions such as diarrhea and pneumonia are associated with poor housing, hygiene and nutrition [2]. Different management and environmental factors such as colostrum feeding, housing, calving assistance, production system, herd size, season and hygiene of microenvironment were reported to affect significantly calf morbidity and mortality. It is estimated that 20% calf mortality resulted in 38% reduction in the profit of a livestock farm. Experience indicated that young animal losses can be significantly reduced by introducing new techniques of management, including proper feeding and nutrition, housing and hygiene [3].

Furthermore, herd replacement can be attained by improved management strategies that can reduce stillbirth and pre-weaning mortalities. Long-term effects of neonatal calf diarrhea(NCD) in dairy heifers include reduced weight gain and development, increased time to first calving and reduced milk production in the first lactation, which result in significant economic losses to the livestock sector [4].

As the leading cause of dairy calf morbidity and mortality, NCD also raises serious concerns on newborn calf welfare and the excessive use of antibiotics with potential increase in antibiotic resistance [5]. Additionally,

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NCD is frequently associated with nutritional and/or immunological factors, such as failure in the transfer of passive immunity and environmental and management factors that either favor the transmission of the causative agents or increase the susceptibility of the calves. Transfer of passive immunity is arguably the single most important non-infectious factor determining neonatal calf health and survival. Calves with failure in the transfer of passive immunity are at increased risk of disease and mortality and a large proportion of calf deaths up to 3 weeks of life can be attributed to failure in the transfer of passive immunity [6].

Calf diarrhea is attributed to both infectious and non-infectious factors. Multiple infectious pathogens such as viruses, bacteria, protozoa etc. are involved in the development of this disease. Co-infection is frequently observed in diarrheic calves although a single primary pathogen can be the cause in some cases. The prevalence of each of pathogen and disease incidence can vary by geographical location of the farms, farm management practices and herd size [7].

The major viral agents that have been implicated in calf diarrhea are Bovine coronavirus (BCV), group A Bovine rotavirus (BRV) and Bovine viral diarrhea virus 1 and 2 as viral agents. Infection with the viruses reduces the absorptive capacity of the intestines because of destruction of enterocytes and disrupts reabsorption of water, thereby leading to loss of fluid and electrolytes [8]. Also there are other non-infectious factors that cause calf diarrhea, such as insufficient uptake of colostrum, poor sanitation, stress and cold weather, could cause neonatal calf diarrhea.

Therefore, the objectives of this review are:

- To review the epidemiology of viral causes of calf diarrhea and its impact on calves.
- To provide information on the effective preventive and control measures for calf diarrhea.

Major viruses that cause calf diarrhea

Rotavirus: Bovine rotavirus is a primary etiological agent of calf diarrhea. The virus belongs to the genus Rotavirus within the family Reoviridae. The genus has 7 species (A to G). Each species especially the species rotavirus A contains different strains and genotypes. Rotavirus is a non-enveloped virion possessing 11 double-stranded RNA segments (16 ◎ 21 kb) and is very stable over a wide pH range with heat lability [9].

Classification and Structures: The Rotavirus is classified into groups, subgroups and serotypes. The group and subgroup specificity is present on the inner capsid VP6. Thus, currently only rotavirus groups A, B and C have been identified as human and animal pathogens, while groups D, E, F and G have only been identified in animals and Estes *et al.* [10]. The Rotavirus structure is 3D capsid protein structures [11]. Similarly, the virus structurally with dsRNA genome and the exact mechanisms of rotavirus genome assortment, packaging and particle assembly await exploration [12].

Pathogenesis: The pathogenesis of the disease is multifactorial it depends on the age of the host and particular viral gene products [13]. The major mechanism appears to be a decreased absorption of salt and water related to selective infection of the absorptive intestinal villous cells, resulting in net fluid secretion. Bovine rotaviruses group A are enteropathogenic agents more commonly associated with neonatal diarrhea in calves up to 30 days old. The main place for rotavirus infection is brush border of villous epithelial cells in the small intestine. Infected cells are rapidly replaced with undifferentiated crypt cells and this result in reducing activity of lactase in villous [14].

Epidemiology of Bovine Rotavirus: Rotavirus causes diarrhea in calves and leads to a serious problem in newly born calves and a cause of economic loss, because of mortality, treatment costs and poor growth. The epidemiology of animal rotaviruses is suggestive and investigated virus infections are those affecting cattle, swine, horse and partly goats, sheep and camelides. Virus distributions is depend on the magnitude of the disease, in developed and developing countries a global survey showed that G1, G2, G3 and G4 were the most common worldwide genotypes of rotaviruses while G8 was relatively high in Africa [15].

Mode of Transmission: The virus is transmitted in interspecies ways with a possibility of cross-species transmission. The interspecies transmission and subsequent reassortments are the important mechanisms driving the diversity of rotaviruses and enabling the emergence of new pathogenic strains with altered virulence [16]. Additionally, it transmitted by fecal-oral route or by direct contact [17].

Diagnosis: Clinical symptoms Rotavirus diarrhea in calves presents an acute disease having a very short incubation period of 12-24 hours or at times ranging from 18-96 hours. Fortunately, most rotavirus infections are mild and self-limiting, although there is usually high morbidity [18]. Variations in clinical disease observed in calves depend on several factors, including the difference in virulence among rotavirus strains, age of the host, host immune status, the dose of the inoculum, occurrence of mixed infections, environmental stress (weather conditions, housing, overcrowding) and nutrition. Laboratory diagnosis of rotavirus is very crucial for handling and preventing the occurrence of the disease in calves. It is challenging to diagnose target causal agents by clinical examination, so that laboratory test is an important tool to make a confirmatory diagnosis. This can be carried out by using various tests [19]. In general, the diagnosis of rotavirus is based on the identification and isolation of the virus in the faces.

Antigen Captured Enzyme-Linked Immunosorbent Assay (Ag-ELISA) is an assay for rapidly detecting a pathogen in a clinical specimen based on antibody (e.g. Monoclonal antibody) recognition of the target antigen [20]. It has antibody attached to a solid surface of polystyrene plate and so that antibody captures the target antigen if present. The enzyme tagged with the secondary antibody can cause a colorimetric visualization while reacting with the chromogen substrate which ensures the antigen-antibody reaction persistence. Antigen can be both qualitatively expressed and quantitatively estimated as Optical Density (OD) measured by a spectrometry positively correlates with the amount of antigen [21].

Virus isolation is a confirmatory diagnostic test that is still considered as “gold standard” for detecting the presence of viral pathogens in specimens [22]. Isolation of rotavirus in cell culture from fecal samples is the most conventional way of confirmatory diagnosis of rotavirus infection and gives the ultimate proof of virus association with the disease but it is less sensitive and is a laborious process. Specimens should be kept at a low temperature and in a transport medium during shipping to a diagnostic laboratory and delivered to the lab as soon as possible after collection [23]. Isolation of BRV is performed in rotavirus- specific primary cell cultures (calf kidney cells) and cell lines (MA 104-Simian origin, MDBK, HT-29 and PK- 15). The presence of virus is suspected by the occurrence of Cytopathic Effect (CPE) including rounding and detachment of cells in cell culture system. Enhancement of CPE is increased by incorporation of trypsin in the medium in few quantities and by the pretreatment of fecal samples with trypsin [24]. Viability of target virus in a specimen is critical for the success of virus isolation.

Polymerase Chain Reaction (PCR) is usually used for detecting rotavirus. It is a thermos cyclic enzymatic amplification of a specific sequence of the target genes using a pair of oligonucleotide primers that hybridize on each cDNA strand of interest region in the genomic sequence. On the other way, rotavirus dsRNA can be detected in clinical specimens by extraction of viral RNA and analysis by electrophoresis on a polyacrylamide gel followed by silver staining. During electrophoresis, the 11 segments of the rotavirus dsRNA, which are negatively charged Molecules; separate according to Yong-Il *et al.* [25]. Patterns of dsRNA can be visualized in the gel by staining with bromide ion, because it forms a stable complex with nucleic acids. The gel can be stored after staining. Migration patterns of the segments of rotavirus dsRNA allow the classification of rotavirus strains into the “short” and “long” electropherotypes [26].

Most of bovine enteric viruses, like BRV are difficult to isolate or propagate in cell culture, but these viruses can be differentiated by their morphology under an electron microscope. This tool can identify and detects the virus based on morphological feature it has. Accordingly, two types of EM methods have been available: direct EM and immune-electron microscopy (IEM), with positive and negative staining techniques used for visualization. The visualization of viruses, particularly non-cultivatable ones, is a major advantage of EM with rapid turnaround [27].

Bovine Corona Virus: Bovine Corona Virus (BCV) is an etiological agent in three clinical syndromes: gastrointestinal diseases in newborn calves (calf diarrhea), winter dysentery in adult cattle and infections of the respiratory and digestive systems of calves known as pneumoenteritis syndrome. There is evidence that BCV is involved in the etiology of the bovine respiratory disease complex affecting calves from 6 to 10 months of age [28].

Pathogenesis: Bovine coronavirus is widespread in cattle of all ages, resulting in economic losses to the beef and dairy industry throughout the world. The virus presence has been confirmed on all continents and seroprevalence

studies demonstrate that over 90% of cattle have been exposed to BCV during their lifetime [29]. Recent evidence suggests that BCV can persist in colostrum-deprived calves or colostrum-fed calves with repeated nasal shedding and a detectable BCV antibody for up to 3 years suggesting that active immune response does not always result in viral clearance [30]. The virus plays a major role in the development of calf diarrhea during the first 3 weeks of life in both dairy and beef cattle herds [31].

Transmission: Bovine coronavirus is transmitted via the fecal-oral or respiratory route. It infects epithelial cells in the respiratory tract and the intestines; the nasal turbinate, trachea and lungs and the villi and crypts of the small and large intestine, respectively [32]. Replication leads to shedding of virus in nasal secretions and in feces. Important factors for the pathogenesis are still not fully explored, such as how the virus infects enterocytes shortly after introduction to an animal. Despite being enveloped, BCVs are relatively resistant pathogens in the environment. Coronaviruses are characterized by a high mutation and recombination rate, which makes host jumping and cross-species transmission easy. In fact, increasing contact between different animal species fosters cross-species transmission, while agriculture intensification, animal trade and herd management are key drivers at the human-animal interface. If contacts with wild animals are still limited, humans have much more contact with farm animals, during breeding, transport, slaughter and food process, making corona virus a persistent threat to both humans and animals [33].

Clinical Signs: Clinical signs begin approximately 2 days after exposure and continue for 3 to 6 days. Typically, coronavirus infection causes profuse watery diarrhea and feces can contain blood clots. Calves become moderately depressed, the suckling reflex is weak and dehydration can develop rapidly. Decreased food intake, fluids and electrolyte loss can result in dehydration, metabolic acidosis and hypoglycemia [34].

Diagnosis: Bovine coronavirus infection can be diagnosed by detection of viral antigen, or viral RNA in tissues, or various animal secretions/excretions. Comprehensive diagnosis includes virus detection in nasal secretions, lung homogenates or feces and virus isolation in HRT-18 cells during the acute phase and/or detection of BCV-binding or virus neutralizing (VN) antibodies in the convalescent phase [35]. BCV antigens are commonly detected by ELISA using BCV MAbs which can be used for fast and reliable analysis of large sample numbers. RT-PCR, nested RT-PCR, real-time qRT-PCR (targeting most conserved genomic regions-ORF1ab or N gene) are the most sensitive assays currently available for BCV detection. For fecal samples, internal controls or additional sample dilution may be needed to detect interference by PCR inhibitors [36].

Minor Viruses That Cause Calf Diarrhea

Bovine Viral Diarrhea Virus: Bovine viral diarrhea virus is an enveloped, positive-sense; single-stranded RNA virus (12.3 kb) and a member of the genus Pestivirus in the family Flaviviridae [37]. The two genotypes of BVDV are known: BVDV-1 and BVDV-2. BVDVs are also grouped into non-cytopathic (NCP) or cytopathic (CP) biotype groups based on their ability to produce defects in cell culture. Pregnant cows and heifers deliver persistently infected (PI) calves if they are exposed to a non-cytopathic BVDV during 45 125 days of gestation since the fetus is not immunocompetent. Most PI calves are born weak and susceptible to other pathogens and experience poor growth. The PI animals also develop fatal "mucosal disease" when exposed to either exogenous or endogenous cytopathic BVD. Mucosal disease is clinically characterized by mucosal ulceration, vesicle formation, erosions, diarrhea and death [38].

Bovine Toro Virus: Bovine Toro virus is a newly identified enveloped, single-stranded, positive-sense RNA virus that belongs to the subfamily Toroviridaein the family Coronaviridae, order Nidovirales. The genome of ToV is 25–30 kb in length and contains two large open reading frames (ORFs) that encode two nonstructural proteins, ORF1a and ORF1ab and four structural proteins, the spike (S), membrane (M), haemagglutinin-esterase (HE) and nucleocapsid (N) proteins [39].

Bovine Norovirus: Noroviruses are non-enveloped RNA viruses that are members of the family Caliciviridae. Based on phylogenetic relationships inferred from the VP1 sequences, noroviruses have been divided into 6 genogroups (GI to GVI), with bovine noroviruses (BNV) classified as GIII [40]. The pathogenesis of BNV is poorly understood; however, extrapolation from other species, especially humans, suggests that BNV can be transmitted via the fecal-oral route, through contaminated food or water [41].

Bovine Nebovirus: Similar to norovirus, Nebovirus is a non-enveloped member of the family Caliciviridae. Infection of gnotobiotic calves with BNeV causes lesions in the jejunum similar to those described for BNV and these lesions have been associated with malabsorption. The mechanism of diarrhea due to BNebV remains poorly understood but malabsorptive and hypersecretory diarrhea can be expected. Clinical signs in gnotobiotic calves infected with BNeV included depression, anorexia and diarrhea [42]. There a tendency for a higher prevalence in calves that are separated from the cow within 24 h after birth compared with those separated later. Nebovirus-positive calves tended to be older than negative ones.

Risk Factors: Calf scours is an outcome of collective interaction between different composite factors intermingled with each other. Host, agent and the environmental factors interacts dynamically with each other leading to the disease occurrence. It is of immense importance to understand the dynamics between different factors to effectively control the calf scours in a herd. There is also an age dependent susceptibility of the calves to the different organisms causing scours [43].

Host: Age of calf is the most important factor affecting mortality. A newborn calf does not receive any antibody from the dam and is very susceptible to environmental pathogens. Calves born from underfed cows have poor growth performance, low productivity and higher susceptibility to disease compared to calves born from cows fed supplemental protein during the last trimester. Differences in susceptibility of calves to diseases are often observed among different breeds [44]. The degree of mortality and morbidity rates were also found with increment trends as blood level of cross bred calves increases. The higher magnitude of mortality and morbidity was recorded from calves of above 75% exotic genetic influence than other percentages of exotic blood level influence [45]. In contrast to this study the breed of calf showed no significant variations in calf mortality rate. Dystocia is closely related to poor calf performance as well as increased susceptibility to environmental pathogens which frequently cause calf diarrhea [46].

Agent: Strain Diversity: the virus is diverse with their antigens. In developed countries a single genotype predominates at a geographic site. However, in developing countries, no single prevailing strains [47]. The genetic evolution of RNA viral pathogens such as BRV, BCoV, BVDV, BToV, BNoV and Nebovirus should be kept in mind and monitored with regular genomic sequence updates. Genetic diversity, continuous evolution, emerging pathogens and/or environmental ubiquity of pathogens are factors that hinder effective control of the disease 48].

Environmental Contamination: Exposure to a contaminated environment is the main cause of calf diarrhea. A simple solution would be to reduce the pathogen load into the environment where calves are raised although this has always been a challenge for cattle producers [49]. After birth, calves are directly exposed to contaminated environments which can be influenced by various factors such as the presence of infected animals, overcrowding, concurrent cow-heifer calving, contaminated calving lots and a lack of calf segregation by age [50].

Temporal Pattern: In some circumstance the occurrence of disease is associated with temporal pattern. The rotavirus infection is occurred during the autumn to spring season. In temperate zone the virus shows seasonal patterns, with the epidemic peaks being more prominent during cold months, but in subtropical and tropical settings, seasonality is less apparent [51].

Economic Importance: Enteric diseases are common and represent huge economic losses to the livestock, meat and milk industry, as a result of newborn mortality and treatment costs. High morbidity and mortality rates in newborn calves are attributed to infectious diseases [52]. Economic losses are not only due to mortality but also due to the cost of treatment and management. In addition, diarrhea has long-term effects on productive and reproductive performance, such as reduction in milk production, reduction in the average daily gain of weight. Digestive disorders in calves are frequent diseases that manifest with diarrhea characterized by liquid and profuse feces, dehydration, wasting, prostration and death [53].

Public Health Importance: Bovine rotavirus is the most recognized pathogen causing acute diarrhea in calves less than one month of age worldwide. It has also been recognized as the major pathogen of acute diarrhea in both humans and animals. So, it has the potential of zoonotic and economic impact [54]. The possibility of interspecies transmission of NoV was demonstrated by a

study in which gnotobiotic pigs were infected with a human NoV strain, raising a concern for the zoonotic potential of this virus worldwide. Groups A to C have been shown to infect both humans and animals [55].

Prevention and Control: There is no specific treatment for viral infections. Treatment is based on providing supportive care and managing clinical signs and potential complications. Fluid administration is essential to replace losses from diarrhea, to correct acidosis and to restore electrolyte imbalance. Adequate sodium concentration and appropriate glucose-to-sodium ratios are the most important components of an efficient rehydration solution [56]. In young animals, administration of fluids can be performed using an esophageal catheter; in older animals, intravenous administration is preferable. Administration of a plasma protein mixture, consisting of immunoglobulin, growth factors and other biologically active peptides, has been advocated to enhance small intestine recovery [57]. Therefore most widely practiced treatments are antiviral therapy, fluid administration and electrolyte management [58].

The colostrum contains antibodies, immune cells (neutrophils, macrophages, T cells and B cells), complements, lactoferrin, insulin-like growth factor-1, transforming growth factor, interferon and other soluble factors as well as nutrients (sugars and fat-soluble vitamins). Immunoglobulins play the most crucial role in the complete development of the calf's immune system [59]. Immunoglobulin G is the primary antibody isotype in bovine colostrum. There are three main classes of immunoglobulins present in colostrum. "IgG, IgA and IgM accounting for approximately 85% to 90%, 5% and 7%, respectively "The relationship between IgG concentrations and calf health is best understood; thus, the concentration of IgG in colostrum is considered the hallmark of evaluating colostrum quality" [60].

The quality of colostrum varies based on calving number, nutritional status and vaccination of the cow. However, calves born to heifers can receive an acceptable level of maternally derived immunity if enough volume of colostrum is ingested within the first 24 h of life [61]. And also within the first 24 hours of life calves should be removed from the mother and housed alone in a clean, dry and warm environment where they can adapt the outside world. Once the calves are moved from their mother, they can suffer by dramatic temperatures changes due to the change in their environment. If calves are immediately removed from their mother's post-partum, infrared heaters for the first 24 hours of life can reduce the dramatic change in temperature. The infrared heaters seemed to have a benefit on the calf's overall health and adaptation to the new environment. The calves under heaters also spent less energy trying to stay warm and more energy improving and developing their respiratory and digestive systems [62].

Having a sustainable housing environment for the calf is beneficial to their thermal, physical and behavioral comfort. Being in a stressful environment can cause predisposition of the calf's health comprising their immune system and affecting their growth rates. Unsafe and frustrating environments can cause stress on the calf resulting in a negative impact on their immune system [63]. Calves should also be housed individually to minimize the spread of diseases and to reduce pathogen transmission. Isolated housing provides easier observation for the calf feeder to maintain the health and provide any necessary medical attention for each individual calf [64].

CONCLUSION AND RECOMMENDATION

Calf diarrhea has been a major disease that negatively affects the cattle industry. The economic impact caused by this condition is significant although many new intervention strategies (e.g., vaccines, medications and herd management) have been developed and implemented to minimize the economic loss. Many enteric pathogens are involved in calf diarrhea, infection and transmission are accomplished via a fecal-oral route. Care must be thus taken to prevent pathogen transmission. The non-infectious risk factors that can cause calf diarrhea must be considered because newborn animals are vulnerable to environmental stresses. The management and control of calf diarrhea before an outbreak is more cost-efficient than treating sick animals after the outbreak occurs. Advice from professional consultants such as veterinarians and nutritionists is necessary to obtain an accurate diagnosis and control or manage risk factors associated with calf diarrhea in modernized large production systems. The use of diagnostic tests has increased the detection frequency of pathogens that were previously neglected. Therefore the following recommendations are suggested:

- Calves should be removed within the first 24 hours of life from the mother and housed alone in a clean, dry and warm environment where they can adapt to the outside world.

- Calves should be left to take an adequate amount of colostrum within the first 24 hours of life.
- The house should be effectively cleaned and disinfected to prevent the introduction of infectious agents.
- Sick calves should be isolated and provided any necessary nutrition and supportive medication and followed up until recover.
- A newborn calf must be vaccinated to prevent and control calf diarrhea.
- Good management and supportive treatment for calves born with infection.
- Besides treating neonatal calf diarrhea, the veterinarian should advise the dairy farmer on how to manage calves and prevent them from calf diarrhea disease.

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