Vets Review

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Progressive Veterinary Doctors Association

Dr. Bidhan Chandra Roy

Vets Review

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March 2016 Vol 1 Issue 1

Editorial Message

Dear Doctor,

It gives me immense pleasure to wish you all our readers/ members and veterinary professionals, A Happy and Prosperous New Year 2016.

The livestock sector in India plays a major contributor to the agricultural economy of our country, not merely in terms of income but also in terms of livelihood and employment. It is usually said that livestock wealth is more equitably distributed than agricultural land. There is an upwards flow of demand for livestock products due to increase in population, growing per capita income and better living standards.

More livestock population means more wealth and more income to the nation and it is one of the major financial supports to the small and marginal farmers in India. "Vets Review" has been an instrumental of knowledge dissemination and sharing up to the field label. I think this is an opportunity for us to renew its usefulness and the value that it will try to provides our readers. I therefore encourage you to write to us with suggestions on how to make it better.

On this hopeful note, we start our journey in the New Year.

With our best wishes
Editors.

WEST BENGAL UNIVERSITY OF ANIMAL AND FISHERY SCIENCES

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Prof. Purnendu Biswas, Ph.D.
Vice-Chancellor

No. : VCS/WBUAFS/ M-5/103
Date : 03.3.2016

MESSAGE

I am extremely happy to learn that the Progressive Veterinary Doctors' Association, Belgachia, Kolkata-37 is going to publish a Technical Bulletin entitled " VETS REVIEW" on 13th March, 2016 at VCI Auditorium, Belgachia on the occasion of its Annual General Meeting.

Publication of such Technical Bulletins keeps the readers updated regarding the latest developments in the field of Veterinary Sciences and helps in skill development. I feel that the Association will be publishing such Technical Bulletins at regular interval.

I am confident that this instant issue of the Technical Bulletin will be quite informative and helpful to the fraternity of Veterinary Sciences as a whole and the Veterinary Practitioners in particular.

I wish you all the best.

(Purnendu Biswas)
VICE CHANCELLOR

Dr. Subal Chandra Patra
General Secretary
Progressive Veterinary Doctors' Association
37, Belgachia Road, Kolkata- 37

Rajesh Kumar Sinha, IAS

सत्यमेव जयते

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No.560/SECT/ARD/16 Dated the 4th March,2016

MESSAGE

I am glad to know that the Progressive Veterinary Doctors' Association is going to publish a technical bulletin "Vets Review" in a befitting manner.

I hope this bulletin will be very helpful to all veterinarians.

I convey my best wishes to all concerned.

(Rajesh Kumar Sinha)

The General Secretary
Progressive Veterinary Doctors' Association

পশ্চিমবঙ্গ সরকার
প্রাণী সম্পদ ও প্রাণী স্বাস্থ্য অধিকার
দূরভাষ ঃ (০৩৩) ২৩৩৫-১১৪৫
মোবাইল ঃ ৯৩৩১২৭৫৫২২
ফ্যাক্স ঃ (০৩৩) ২৩৩৫-১১৮৭
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MESSAGE

I am delighted to learn that the Progressive Veterinary Doctors Association has taken an initiative to publish a Technical Bulletin namely "VETS REVIEW" on 13th March, 2016 at Veterinary Council Auditorium, Belgachia.

I sincerely hope that it would be a good quality scientific publication along with a focus on field oriented problems which would update the knowledge and promote the professional efficiency of the veterinarians.

I extend my best wishes for the success of the Technical Bulletin on its maiden publication.

3.3.16
(Dr. S. Bose)

Director of Animal Husbandry &
Veterinary Services, West Bengal

To

The General Secretary,
Progressive Veterinary Doctors Association.

কার্য্যালয় ঃ প্রাণী সম্পদ ভবন, তৃতীয় তল, এল.বি.-২ ব্লক, সেক্টর-৩, লবণ হ্রদ, কলকাতা-৭০০ ০৯৮
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Prof. Ashis Kr. Samanta
Registrar

Ref. No. WBUAFS / Estb./ M-1 (Pt.II) / 529

Date: 04.03.2016

MESSAGE

I convey sparkling wishes to the "Progressive Veterinary Doctors' Association" for their endeavour in publishing a technical bulletin - '**Vets Review**'. The 'Vets Review' would primarily aim at the veterinarians; therefore, the kind patronage of all the veterinarians is essentially required for the bulletin as readers. In pursuance of my professional understanding, I would suggest that 'Vets Review' should depict the experience of the veterinarians in their practicing field both at lab and land situations. The bulletin should encourage educating and disseminating information on animal well-being, their diseases, management etc. and come up with newer and latest information for the practitioners, livestock and poultry keepers, breeders as well as the industry personnel.

I pass on good wishes and appreciate the commendable venture of the "Progressive Veterinary Doctors' Association" for release of the technical bulletin.

I passionately hope to see the continued publications of the 'Vets Review'.

(A. K. Samanta) 4/3/16
Registrar (Actg.)

To
Dr. Subal Chandra Patra,
General Secretary,
Progressive Veterinary Doctors' Association,
37, Belgachia Road, Kolkata – 700037.

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PREFACE

Our State vis-a-vis our Country has large populations of various species of livestock and poultry and majority of which are suffering from different diseases and among these diarrhoeal diseases play an important role and impose a huge national loss by way of morbidity and mortality. The losses due to diarrhoeal diseases can be drastically reduced by adopting appropriate and timely measures for accurate diagnosis and treatment.

Under the changed global scenario the veterinarians have to be fully equipped, knowledgeable and skilled in latest disease situation and diagnostic methodologies. During recent past calf diarrhoea got manifested into the state due the several aetiological factors and global climatological changes.

The primary objective of our Technical Bulletin ***"Vets Review"*** *is to upgrade the knowledge and skill of veterinarians towards efficient and effective diagnosis and treatment.*

Considering this perspective, we are going to publish our First Technical Bulletin ***"Vets Review"*** *for providing information to policymakers, scientists, veterinarians, students and general public and which ultimately boost up the knowledge of veterinarians towards development of rural economy.*

We feel and believe that our beloved veterinarians holding different responsible chairs including laboratories, dispensaries, administration, teaching, research and development and extension activities will be able to disseminate their knowledge properly.

All are cordially welcome for constructive criticism.

With thanks

Subal Chandra Patra

(Dr Subal Chandra Patra)
General Secretary
Progressive Veterinary Doctor's Association

An Overview of Calf Diarrhea (Scours)

Introduction

Diarrhea in neonatal calves is one of the most challenging clinical syndromes encountered by practicing large animal's veterinary practitioners. The objectives are to study the prevalence of diarrhea cases in cattle and buffalo calves and, their treatment & control.

Calf diarrhea (also known as calf scouring) is a commonly reported disease which is generally occurred birth to 14 days or even up to 30 days and characterized by watery, yellowish-green, foetid-adheres around tail-dehydration-heavy death rate-sometimes naval ill and arthritis (Fig: 1, 2, 3, 4). It is one of the major cause of economic loss to cattle producer. It has been reported that 57% of weaning calf mortality was due to diarrhea and most cases occurred in calves less than 1 month old. (Walker et al., 2012)

Calf diarrhea is attributed to both infectious and non-infectious factors. Multiple enteric pathogens (e.g., viruses, bacteria, and protozoa) are involved in the development of this disease (Uhde et al., 2008). 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 animals along with their management practices.

Although the cattle industry has made great improvements with herd management, animal facilities and care, feeding and nutrition, and timely use of bio-pharmaceutics, calf diarrhea is still problematic due to the multi-factorial nature of the disease. Prevention and control of calf diarrhea should be based on a good understanding of the disease complexities such as multiple pathogens, co-infection, environmental factors, and feeding and management during the calving period before disease outbreaks. In this overview, infectious agents involved in calf diarrhea, appropriate application of diagnostic methods for identifying these pathogens, and intervention strategies for managing calf diarrhea are described.

Fig. 1 Buffalo calf with diarrheic motion.

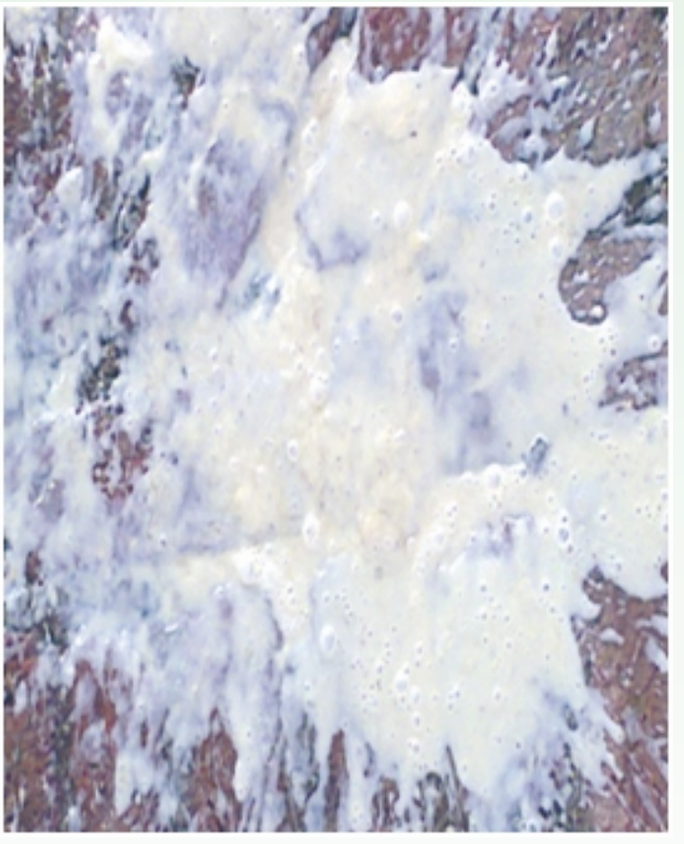

Fig. 2 Diarrheic faces

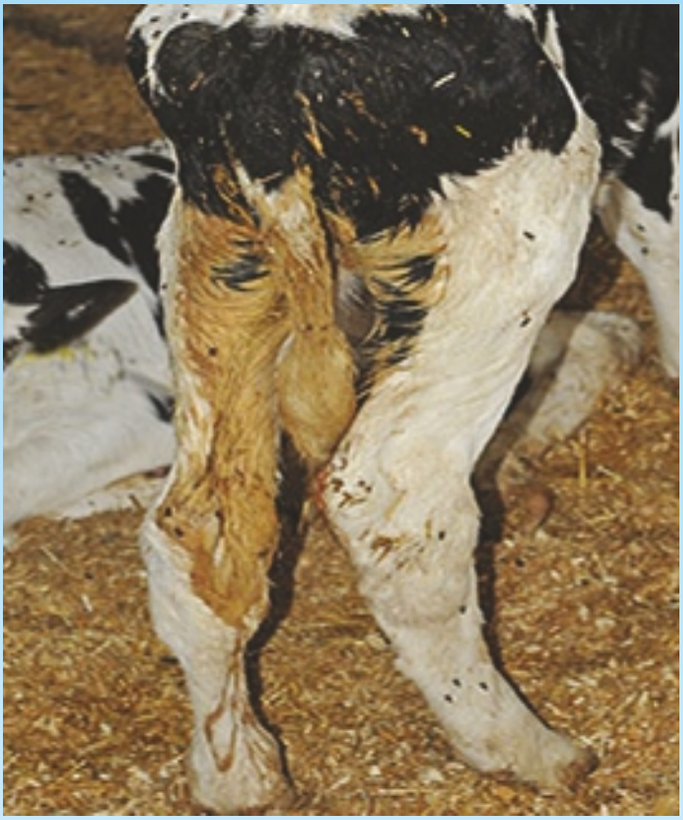

Fig. 3 Diarrheic calf with arthritis

Fig. 4 Diarrheic calf with dehydration

Etiologies:

Numerous infectious agents have been implicated in calf diarrhea. Bovine practitioners and cattle producers are aware of many enteric pathogens because these primary agents have been known to be involved in calf diarrhea for several decades and still greatly influence current cow-calf operations. Ten different enteric pathogens are recognized as either major (BRV, BCoV, BVDV, Salmonella spp, E. coli, C. perfringens, and C. parvum) or emerging (bovine caliciviruses and BToV) pathogens (Uhde et al., 2008; Cho et al., 2013). Characteristics of different enteric pathogens (viruses, bacteria, and protozoa) including more recent findings are briefly described below.

Viruses: One of the main causes is viral infection. Bovine rotavirus is a primary etiological agent of calf diarrhea. The virus belongs to the genus Rotavirus within the family Reoviridae. 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. There are seven serogroups (A through G) of rotaviruses based on antigenic and genetic similarities of the intermediate capsid protein (VP6). Group A rotaviruses are the major cause of rotaviral infection in domestic animals. Most BRVs (95%) belong to group A, although groups B and C rotaviruses have also been identified in field cases.

Group A rotaviruses can be further classified into P or G types based on genetic and antigenic similarities of VP4 (protease sensitive protein) and VP7 (glycoprotein) which constitute the outer capsid of the virion and induce anti-viral neutralizing antibody production. Sixteen G types and 27 P types have been reported in domestic animals. Bovine rotaviruses are G1, G6, G8, or G10 types. G6 and G10 type are reported to be the most prevalent in cattle.

While VP4, VP6, and VP7 play a major role in maintaining viral structure, virus attachment, and antigenicity, nonstructural glycoprotein 4 (NSP4) holds a special role as a viral enterotoxin. This protein also interferes with cellular homeostasis by elevating calcium ion influx into the cytoplasm. These alterations account for drastic changes in the movement of nutrients and water across the intestinal epithelium and are more important for viral pathogenesis than histopathological lesions.

Bovine rotavirus usually causes diarrhea in calves at 1 to 2 weeks of age. The milk uptaken by calves can provide a good environment for rotavirus survival under a wide range of gastrointestinal pH levels and infection of the intestinal

epithelial cells. This may explain why weaning calves are more susceptible to calf diarrhea. The virus has a very short incubation period (12~24 h) and induces peracute diarrhea in affected calves. Once infected, the calves shed a large amount of virus via faeces for 5~7 days, thus contaminating the environment and allowing the virus to be transmitted to pen mates. The virus replicates in the cytoplasm of epithelial cells of small intestinal villi. Destruction of mature enterocytes in the villi, activation of the enteric nervous system by vasoactive components from the damaged cells, and secretion of a viral enterotoxin (e.g., NSP4) account for maldigestive/malabsorptive diarrhea promoted by rotavirus infection. Viral infection causes villus atrophy and usually affects the caudal part of the small intestine. Evidence for interspecies transmission along with genetic reassortment between human and animal rotaviruses (e.g., swine, bovine, feline, and canine) has raised concerns about zoonotic rotaviruses.

Bovine coronavirus is an enveloped virus with a positive-sense, single-stranded RNA genome (27~32 kb). This pathogen is a member (Betacoronavirus 1) of the genus Betacoronavirus that was formerly classified as group 2a coronaviruses. Virus infection can present as three distinct clinical syndromes in cattle: a) calf diarrhea in calves at 1 to 2 weeks of age; b) winter dysentery with hemorrhagic diarrhea in adult animals; and c) respiratory diseases including bovine respiratory disease complex in both young and adult cattle.

Viral infection begins in the small intestine and usually spreads through the entire small intestine and colon. Microscopically, villi of the affected small intestine and colonic crypts become atrophic, and the lamina propria becomes necrotic. Initially, the S protein and hemagglutinin-esterase (HE) protein of the virus attach and fuse to the intestinal epithelial cells. . The virus replicates in enterocytes and progeny viruses are released through a normal secretory mechanism and cell lysis. Mature villous epithelial cells are the primary target of the virus although crypt enterocytes are also affected. Clinical signs in affected animals often have a longer duration due to the damage done to crypt enterocytes by the virus.

Bovine viral diarrhea virus is an envelope, positive-sense, single-stranded RNA virus (12.3 kb) and a member of the genus Pestivirus in the family Flaviviridae . There are three species included in the genus: BVDV, border disease virus, and classical swine fever virus. BVDV can be divided into two types (BVDV1 and BVDV2) based on sequence similarity of the 5' untranslated region (UTR) in the viral genome. In addition to these two types, BVDV3 was recently proposed as tentative species together with other Pestivirus species (e.g., border disease virus type 2, Pronghorn, and Bungowannah). Each type can be further divided into two biotypes (cytopathic and noncytopathic) based on their ability to cause lytic cytopathic effects in cell culture. Noncytopathic strains of BVDV are responsible for persistent infection of the virus in cattle. To date, 15 (BVDV1a to BVDV 1o) BVDV1 and two (BVDV2a and BVDV2b) BVDV2 subgenotypes have been recognized. BVDV1a, BVDV1b, and BVDV2a are the most prevalent subgenotypes in cattle populations. BVDV1c is the most common subgenotype in Australia.

The clinical symptoms of BVDV infection vary from subclinical to fatal disease depending upon host immune status, pregnancy and gestation period, and the presence or absence of co-infection with other pathogens. Most infected animals develop mild clinical signs such as low-grade fever, leukopenia, anorexia, and decreased milk production. Acute BVD infection is characterized by diarrhea, pyrexia, depression, anorexia, decreased milk production, oral ulcerations, hemorrhagic syndrome, and lymphopenia/leucopenia leading to immunosuppression. Immunosuppressed cattle become susceptible to other diseases due to the concurrent infection with other pathogens (e.g., bovine respiratory disease complex). Although most immunocompetent animals eventually clear the virus and recover from the disease, some infected cattle occasionally harbor the virus for a long time with periodical appearance of transiently detectable viremia from time to time (e.g., transiently infected animals).

Pregnant cows and heifers deliver persistently infected (PI) calves if they are exposed to a noncytopathic 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 BVDV. Mucosal disease is clinically characterized by mucosal ulceration, vesicle formation, erosions, diarrhea, and death. BVDV can cause calf diarrhea in two major ways: 1) persistent infection resulting in primary damage to enterocytes and susceptibility to co-infection, or 2) transient infection with replication in crypt enterocytes and lesion formation contributing to diarrhea.

Bovine torovirus is an envelope, positive-stranded, RNA virus (25~30 kb) belonging to the genus Torovirus in the family of Coronaviridae, order Nidovirales along with equine torovirus, porcine torovirus, and human torovirus. Toroviruses are infectious gastrointestinal agents in cattle, and a predominant cause of acute enteric infection in piglets and children. Fecal shedding of BToVs from diarrheic

calves has been reported around the world including the USA (2003, 2002, 1983, and 1982), Canada (1998), Costa Rica (1998), Korea (2008), the Netherlands (1991), Germany (1992), Hungary (2002), Austria (2006), Asia (2007), and South Africa (1993). Morphological similarities and antigenic cross-reactivity between human and bovine toroviruses has raised a concern about the potential zoonotic nature of BToV.

Noroviruses are a major cause of acute and sporadic non-bacterial gastroenteritis in humans (both adults and children). These pathogens have also been reported to cause gastroenteric disease in animals such as cattle, pigs, dogs, and mink. Recently, an experimental challenge study with the Jena strain of BNoV was conducted on newborn calves infected via an oral route. The investigators demonstrated that the virus infected epithelial cells of the small intestine and caused villous atrophy (in the jejunum and ileum) leading to diarrhea with virus shedding but not seroconversion. Detection of BNoV in feces from clinically healthy cattle has also been reported, raising questions about the clinical significance of BNoV.

Bacteria: The most common bacteria causing calf diarrhea is Salmonella enteric, which colonizes the gastrointestinal tract of a wide range of hosts. S. enterica serovar Typhimurium (S. typhimurium) and serovar Dublin (S. dublin) are the most common etiologic agents that cause salmonellosis in cattle. S. typhimurium is the most common serotype that affects calves in the India.

Salmonella infection has a wide variety of clinical symptoms ranging from asymptomatic to clinical salmonellosis. Acute diarrheal disease is most common with S. typhimurium and systemic disease is associated with S. dublin. Calves less than 3 weeks of age are commonly infected by Salmonella. The lesions frequently observed in affected calves involve the pseudo membrane on the mucosa of the small intestine as well as enlargement of the mesenteric lymph nodes. Infected cattle can serve as a source of zoonosis through food-borne routes or direct contact.

The basic mechanism underlying Salmonella virulence includes the ability to invade the intestinal mucosa, multiply in lymphoid tissues, and evade host defense systems, leading to systemic disease. For Salmonella pathogenesis, the organism should be capable of invading intestinal epithelial cells, surviving within macrophages, and causing enteropathogenicity. Salmonella colonizes M-cells, enterocytes, and tonsilar tissues. Following lymphoid tissue (e.g., tonsilar tissue) infection, Salmonella easily spreads throughout the whole body by invading mononuclear cells and phagocytes. Salmonella pathogenicity island 1 (SPI-1) and SPI-5 are known to influence the type III secretion system, and are mainly responsible for Salmonella-induced diarrhea in calves. SPI-2 is involved in the second type III secretion system and is responsible for intracellular survival of the organism.

Clinical presentation of salmonellosis is characterized by watery and mucoid diarrhea with the presence of fibrin and blood. Even though Salmonella can cause diarrhea in both adult cattle and calves, infection is much more common and often causes severe symptoms in 10-day to 3-month old calves. Calves can shed the organism for variable periods of time and intermittently depending on the degree of infection (e.g., clinical or subclinical infection).

The second most common is Escherichia coli and it can be classified into six pathogroups based on virulence scheme: enterotoxigenic E. coli (ETEC), shiga toxin-producing E. coli, enteropathogenic E. coli, enteroinvasive E. coli, enteroaggresive E. coli, and enterohaemorrhagic E. coli. Among these bacteria, the most common cause of neonatal diarrhea is ETEC stains that produce the K99 (F5) adhesion antigen (commonly referred to as E. coli K99+) and heat-stable enterotoxin. It should be noted that other pathogroups of E. coli, which are usually identified by histopathology, can be missed if the diagnosis focuses on E. coli K99+ alone.

Neonatal calves are most susceptible to ETEC infection during first 4 days after birth and develop watery diarrhea if infected. Following ingestion, ETEC infects the gut epithelium and multiplies in enterocytes of the intestinal villi. The distal portion of the small intestine provides the most favorable environment for ETEC colonization due to the low pH (less than 6.5). Villous atrophy due to a loss of infected cells and damage to the laminar propria are commonly observed in affected small intestine. The bacteria express the K99 antigen for attachment. After colonization of the gut epithelium, heat-stable toxin production induced by ETEC leads to the up-regulation of chloride secretion into the gut. This osmotically pulls water into the intestinal lumen and leads to the development of secretory diarrhea in calves.

The third one, Clostridium perfringens is Gram-positive, spore forming anaerobic bacterium that causes a wide range of diseases in mammals and birds. These microorganisms can be subdivided into five toxin types (A, B, C, D, and E) based on the production of four major toxins: alpha (α), beta (β), epsilon (ε), and iota (ι). Type A strains produce α toxin alone, type B stains produce α, β, and ε toxins; type C strains manufacture α and β toxins; type D

strains secrete α and ε toxins; and type E strains produce α and ι toxins. Among these groups, type C has been frequently reported in conjunction with calf diarrhea but not as common as some other enteric pathogens such as BRV, BCoV, E. coli, Salmonella spp., and C. parvum.

The toxin is the main lethal toxin and promotes cell lysis through the hydrolysis of membrane phospholipids. The β toxin is highly trypsin-sensitive and induces mucosal necrosis. The ε toxin causes lethal enterotoxemia in domestic animals, and the ι toxin is responsible for dermonecrosis due to its high vascular permeability. Enterotoxin causes diarrhea and intestinal cramping due to its effects on epithelial tight junction protein. Beta-2 toxin, which is produced by all types of C. perfringens, has been recently postulated to synergistically function with enterotoxin.

Most domestic animals are susceptible to all types of C. perfringens due to the ubiquitous nature of the bacterium in the environment. Newborn calves which produce a low level of proteolytic enzymes (e.g., trypsin) in the gastrointestinal tract can be easily infected by C. perfringens type C since β toxin is recognized as the main virulence factor responsible for clinical signs seen in animals affected by this bacterium. Intestinal lesions in these infected animals are characterized by diffuse or multifocal hemorrhagic necrotizing enteritis and bloody fluid distension.

Protozoa:

Cryptosporidium parvum is a protozoan parasite that is frequently associated with gastrointestinal tract disease in humans and neonatal cattle. Calves infected with C. parvum can be asymptomatic or develop severe diarrhea with dehydration. There are approximately 24 species of Cryptosporidium. Cattle are commonly infected by C. parvum, C. bovis, C. ryanae, and C. andersoni. C. parvum is considered to be primary cause of calf diarrhea and is a potential zoonotic agent.

Once C. parvum is ingested, the oocyst excystation releases sporozoites that penetrate enterocytes. The excysted parasites undergo asexual (type I meront) and sexual (type II meront) reproduction to produce macrogametocytes and microgametocytes. Upon fertilization of the macrogametocytes by microgametes, zygotes are developed with sporulates (sporogony) generating thin-walled oocysts involved in autoinfection. Next, thick-walled oocysts pass out of the host. The oocysts can survive for more than a month in the environment under favorable conditions (e.g., high temperature and moisture with low UV radiation) and are resistant to most disinfectants. Environments contaminated with oocysts can be an immediate source of infection for both animals and humans. The invasion of C. parvum into enterocytes induces changes in intestinal cytoskeleton structures, such as loss of microvilli and shortening of columnar epithelial cells, leading to severe villous atrophy in infected animal Damage to the intestinal epithelium causes prolonged malnutrition and reduced growth rates in affected calves due to malabsorption and fermentation of undigested milk in the intestinal lumen These result in considerable economic losses in cow-calf production.

Coccidiosis: Coccidiosis sp. mainly Eimeria bovis & Eimeria zuernii can be a very serious disease in weaned calves, but it creates problem mainly in young calves. It is associated with poor sanitation, overcrowding, or sudden changes in feed. Occasionally, affected calves may exhibit signs of brain damage but tarry or bloody scours are commonly observed.

Yeast and Molds: Yeast and molds are sometimes associated with lesions in the stomach or intestines of scouring calves. They are generally found when scouring calves are subject to overuse of antibiotics.

Nutritional Scours: Nutritional scour can be caused by anything that disrupts the normal habit, like over feeding or over consume of milk. This type of scours usually presents little problem. Many of these calves still active and alert, do not require any treatment.

Diagnosis of Calf Enteric Pathogens:

Diarrhea can be fatal to neonatal calves due to dehydration and acidosis that may result in anorexia and ataxia. Since various pathogens or factors have been implicated in the development of diarrheic disease, laboratory testing is necessary for accurate assessment of the problem (e.g., accurate diagnosis). The progression of diarrhea can be rapid. Hence, a quick diagnosis is critical for not only quickly confirming the cause but also helping clinicians and cattle producers to implement appropriate interventions in a timely manner. It should be noted that the diagnostic outcomes can be influenced by many factors such as sampling time and population, types and quality of the specimens, and laboratory methods used. Each of these factors is discussed below.

Procedures for diagnosis of calf diarrhea:

Clinical (e.g., age, vaccination record, and clinical signs) and history should be provided to clinicians for determining the cause of diarrhea. Once the specimens are submitted to a veterinary diagnostic laboratory, the diagnostician sorts the samples to ensure proper delivery to testing laboratories

based on the history and sample type. Generally, faecal sample are examined by microscopy (for C. parvum and Coccidia), bacterial culturing (for Salmonella spp., E. coli, and C. perfringens), and PCR (for BRV and BCoV). In contrast, intestinal tissues are subjected to immunohistochemistry or bacterial culturing. More recently, nucleic acid-based techniques such as PCR and an antigen-capturing enzyme-linked immunosorbent assay (Ag-ELISA) have been more commonly used for the rapid detection of various bacterial and viral pathogens in clinical specimens from diarrheic calves (Amer et al., 2011). When the laboratory test results are available, clinicians should consider the overall farm and clinical history in conjunction with lab results before identifying the causative pathogen.

Laboratory Diagonosis

Laboratory methods for identifying enteric pathogens have typically included pathogen isolation and characterization along with histopathology as the gold standard for etiologic agent and disease confirmation. However, many enteric pathogens are difficult to isolate from the gastrointestinal environment. Direct visualization (e.g., light microscopy or electron microscopy [EM]) of pathogens in feces or intestinal contents as well as the detection of antigens (e.g., Ag-ELISA) or nucleic acids (e.g., PCR) in specimens have been widely accepted as alternative methods.

The virus isolation test is still considered the 'gold standard' for detecting viral pathogens in specimens although new methods such as an ELISA and PCR-based tests have been developed.

Certain viruses due to variations in viral susceptibility of the different cells. Electron microscopy is commonly used for virus detection and identification based on morphological characteristics.

Fecal flotation and direct microscopy are commonly used to diagnose parasite eggs or oocysts, which may be applied in the field condition. The principal of fecal flotation is simply based on the density difference between a flotation solution (≥1.24) and oocysts (1.05~1.24). A centrifugation step is commonly included in the testing procedure to increase detection sensitivity since centrifugation concentrates the target for easy viewing under a microscope. Direct microscopy can also be performed for fecal smears without centrifugation.

Oocysts in clinical specimens may be difficult to visualize without special staining. C. parvum oocysts are reported to be positive for acid-fast staining. Modified acid-fast stains are applied to fecal smears to detect these organisms. Unlike the Ziehl-Neelsen modified acid-fast stain, the modified Kinyoun acid-fast stain contains a more concentrated fuchsin dye and lipid solvent, and does not require heating the reagents used for staining. In brief, one to two drops of feces is smeared on a clean glass slide and air-dried. The sample is fixed with absolute methanol, and subsequently stained with carbol fuchsin and 1% sulfuric acid. The specimen is then counterstained with methylene blue or brilliant green and examined under a light microscope with oil immersion. The red or purple stained C. parvum oocysts 4 to 6 µm in diameter should appear against a blue or green background. This modified acid-fast staining method is widely used to detect C. parvum in feces. The sensitivity of this technique is low because the procedure requires approximately 500,000 oocysts per 1 g of feces to confirm the presence of C. parvum oocysts.

Fecal bacteria culturing is a commonly used laboratory method for isolating and identifying bacterial pathogens in feces and intestinal contents. Salmonella spp., E. coli K99+, and C. perfringens are primary bovine enteric pathogens. In order to prevent any cross-contamination or loss of viability, feces should be collected directly from diarrheic calves by either rectal swabs or rectal stimulation. Once collected, the fecal samples should be stored in a transport medium or special stool container in a cooler or on ice before submission to a diagnostic lab. To examine anaerobic bacteria-like C. perfringens, fecal samples must be immediately stored in a pre-reduced (e.g., oxygen-free) transport medium if available.

Blood agar plates, McConkey agar plates, McConkey agar with sorbitol, Hektoen enteric (HE) plates, and xylose lysine desoxycholate (XLD) plates are used for bacterial culture. Several kinds of enriched and selective media such as brain heart infusion (BHI) broth (a highly nutritious medium for general bacterial culture) and tetrathionate broth (for Salmonella spp.) are employed for growing and identifying certain bacterial pathogens. Blood agar is most commonly used because the majority of bacteria can grow on this medium. MacConkey agar is selectively used to culture Gram-negative bacilli that are commonly present in the gastrointestinal tract and differentiate bacteria that ferment lactose. Sorbitol-MacConkey agar can help distinguish nonpathogenic E. coli from E. coli O157:H7 which cannot ferment sorbitol. Salmonella spp. are typically cultured from fecal samples using Samonell-Shigella agar, bismuth sulfite agar, HE medium, brilliant green agar, and XLD agar. For C. perfringens culturing, thioglycolate broth growth medium is commonly used. Culturing usually takes 2 days at 36oC under anaerobic conditions. Colony morphology (e.g., shape, surface, and elevation of colonies on the agar plates), physical characteristics of the bacteria (e.g., aerobe, anaerobe, or microaerophile), microscopic features (e.g.,

rods, cocci, or coccobacilli), and biochemical tests (e.g., ones that confirm fermentation, gelatin or urea utilization; indole, oxidase, or catalase production, etc.) are then used to characterize and identify the isolated bacteria. Slow turnaround of the results (growth and identification can take 24~72 h) is a disadvantage of bacterial culture tests although the turnaround can vary depending on culture methods and diagnostic instrumentation. In some cases, further immunological testing (e.g., an agglutination test) is required for the identification (e.g., for E. coli K99+) or serotyping (e.g., for Salmonella spp.) of bacteria. A nucleic acid-based assay is also required for typing (e.g., for C. perfringens toxin type).

Prevention and Control of Calf Diarrhea:

Calf diarrhea is a multifactorial disease. Factors involved in the occurrence of calf diarrhea can be summarized as ones associated with a) peripartum calving management, b) calf immunity, and c) environmental stress or contamination. Characteristics of major or emerging bovine enteric pathogens were previously described in this review. There is not much of difference between the patterns of disease development and prevention of calf diarrhea according to each etiological agent. Knowing of causal pathogen(s) is important for accurately assessing the current status of the affected farm and developing further interventions. Nowadays, disease control and prevention in production animals involves animal welfare from the public or consumer's point of view, and increased productivity from the livestock producer's point of view.

Peripartum calving management

Cow nutrition is closely associated with weak labor, amount of milk production, dystocia, and calf growth. Inadequate feed intake and macro- or micro-nutrient deficiencies during the last trimester increase calf morbidity and mortality rates because most fetal growth occurs during last 2 months of gestation. Recently, cow nutrition has been shown to impact the transition of the calf into adult life as well as fetal growth and development. Calves born to underfed cows have poor growth performance, low productivity, and higher susceptibility to disease. In another study, heifer calves born to cows fed supplemental protein during the last trimester were found to have greater pregnancy performance later in life compared to the control group.

Dystocia is closely related to poor calf performance as well as increased susceptibility to environmental pathogens which frequently cause calf diarrhea. Calves that experience dystocia may have physical symptoms such as congestion and swelling of the head and tongue, which can reduce the amount of colostrum uptake from the dam. The absorption rate of colostrum-derived immunoglobulin is lower in these calves compared to healthy animals. Consequently, the affected calves cannot obtain appropriate passive immunity from the dams due to inadequate colostrum uptake during early life (e.g., 2~ 6 h after birth).

Immunity

The bovine placenta does not permit the passive transfer of antibody to the fetus. As a result, the newborn calf does not receive any antibody from the dam and is very susceptible to environmental pathogens. Resistance of the calf to enteric disease is closely related to the timely consumption of high-quality colostrum in sufficient quantities. The neonatal calf should ideally receive 2~3 L (for beef calves) or 3~4 L (in dairy calves) of colostrum within the first 6 h after birth. 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). Immunoglobulin G is the primary antibody isotype in bovine colostrum.

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. Heifers have a greater likelihood for dystocia, mis-mothering, and poor colostrum production compared to a multiparous cow. Therefore, cow-calf management practices (e.g., calving heifers first and segregation of calves based on birth date) should be considered for reducing the chances of infectious disease development.

The primary function of colostrum is to enhance the calf's immune system through the passive transfer of both antibody and cell-mediated immunity. Ideally, calves should receive colostrum from their dams although colostrum from several cows is often mixed and administered or purchased. One caution of colostrum feeding is the transmission of BVDV, bovine leukemia virus, and Johne's disease that can be spread by infected or purchased colostrum. In particular, Mycobacterium avium paratuberculosis (Johne's disease) transmission is the number one risk factor associated with colostrum acquired from dairy cattle and administered to beef cattle. Therefore, colostrum from dairy farms of unknown infection status should be avoided. It is recommended that supplemental colostrum should be obtained from the farm of origin or a historically disease-free facility.

Environmental stress and contamination

Harsh weather conditions such as low temperatures, rain, heavy snow, wind, and high levels of moisture act as stress factors to young calves and increase the susceptibility of calves to diarrhea. Neonatal calves are not able to effectively regulate their body temperature when exposed to extreme weather conditions. This may induce hypothermia or hyperthermia resulting in immune system impairment. The dam is less influenced by environmental stress than the calf. However, the probability of dystocia or metabolic disease is still increased by environmental stress. Special care is required to reduce environmental risk factors closely associated with calving season including the provision of dry, draft-free shelter. The calving season can be adjusted to a time when environmental conditions are more favorable by implementing a controlled breeding program.

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. 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. These factors usually work synergistically and increase the opportunity for increased duration of exposure to a higher quantity of pathogens. Conversely, intervention for preventing calf diarrhea is focused on the control and reduction of each factor (e.g., pathogen load and environment contamination). The basic concepts of intervention for reducing the incidence of calf diarrhea are based on 1) decreasing pathogen exposure by planning to breed and heifers first calving, which reduces the exposure of more susceptible newborn calves to pathogens, 2) reducing pathogen loading into the environment by shortening the calving season through scheduling breeding, which reduces the period of pathogen entry into the environment; and 3) keeping a clean area (or pathogen-free area) by grouping animals according to their calving date so that the calving area can be kept clean after occupation by the previous calving group.

Treatment: The following treatment schedule should be applied along with fluid therapy: Calf scours/diarrhea of virus origin has no treatment, but to check the secondary infection, antimicrobial agents may be given as treatment schedule of bacterial origin. For Bacterial origin: i) Full dose of wide spectrum of Antibiotics such as Amoxicillin @10mg/kg body weight twice daily orally for 5 days.

ii) Amikacin@10mg/kg, I/M two times daily for 5 days

iii) Ceftiofur @5mg/kg I/M daily for 5 days

iv) Electrolytes infusion by slow i/v drip as per degree of dehydration.

For protozoan origin:

i) Cryptosporidiasis-Azithromycin@30mg/kg once daily orally for 7 days.

ii) Coocidiosis-Sulfadimidine (33.3%) -100mg/kg. S/C or orally (tablet/bolus) daily for 3 days, trimethoprim-sulphadoxine (co-trimazine)@15mg/kg, S/C, I/M or slow I/V daily or alternate days for 3 days.

iii) Giardiasis:-Fenbendazole @5mg/kg orally daily for 3 days, or albendazole 20mg/kg orally daily for 3 days, or Metronidazole@25mg/kg I/V daily for 3 days.

Use of Probiotics: The probiotics is very much useful such as bacterial and fungal species include Enterococcus faecium, Lactobacillus rhamnosus, Lactobacillus acidophilus etc. to check the calf diarrhea. The mechanism of action of probiotics are competition for receptor site on the intestinal surface, immune system stimulation, excretion of antimicrobial substances and competition with pathogens for intraluminal nutrients.

Use of Intestinal Protectants: Intestinal protectants like bismuth subsalicylate, kaolin or pectin and activated charcoal can be administered in addition to specific therapy to neutralize the bacterial toxin and reduce the intestinal secretion.

Use of Vitamin A: Enteric absorption of Vit-A is diminished specially in calves with cryptosporidiosis, so systemic use of Vit-A is always desirable.

Guideline for Assessing Dehydration in Neonatal Calves

% of dehydration	Eyeball sunkenness	Neck skin tent (seconds)	Mucous membrane
0	none	<1	Moist
1-5	slight	1-4	Moist
10	Gap between eyeball & orbit	5-10	Tacky
20	Wide gap	50	dry

Calculation the Volume of Fluid (Litre) required=% of dehydration × calf body weight (kg). The Fluids were: 0.9% saline, 1.3% sodium bicarbonate, ringers's lactate and 5% dextrose.

Calculation of Bicarbonate requirement in calf in relation to body weight and severity of acidosis:

Calf weight(kg)	1.3% NaHCO3 in litre
30	0.5
35	0.5
40	0.6
45	0.7
50	0.8
55	0.9
60	1

Summary & Conclusion:

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., vaccine, medications, and herd management) have been developed and implemented to minimize the economic loss. At this present time, the need to make a definite diagnosis to control the disease by using antimicrobial agents and prevented by vaccination.

There are also public health implications to the diagnosis of cryptosporidiosis and salmonellosis. New vaccine may help to control rotavirus and corona virus infection. Treatment of diarrhea in neonates is primarily based on corrective dehydration and acidosis through the use of oral and I/V electrolytes. In the case of bacterial infection antimicrobial agents used to check the bateremias.

Colostrum feeding will help the reduce diarrhea in the first few days of life. Management is the prime important to control the disease.

In approaches the neonatal death losses, the areas to examine should include calf immunoglobulin status (organized Herd), calf feeding, calf housing, cleaniness of environment, calving area, cow vaccinations, diagnosis of specific agents and treatment protocol.

Persistence of this significant problem in the field may be attributed to the multifactorial nature of calf diarrhea including permutations of infectious diseases, a failure to clearly understand the disease ecology, poor environmental hygiene, and biased epidemiological data. Genetic diversity, continuous evolution, emerging pathogens, and/or environmental ubiquity of pathogens are factors that hinder effective control of the disease. Therefore, 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.

Non-infectious risk factors have frequently been neglected by cattle farmers, and also are to be considered equally important as infectious factors because the 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. Although many enteric pathogens are involved in calf diarrhea, infection and transmission is accomplished via a fecal-oral route. Care must be thus taken to prevent pathogen transmission. 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.

In summary, the effective control of calf diarrhea should be based on three major points. First, a clear understanding of pathogen characteristics (e.g., mechanism underlying pathogenicity, prevalence in the field, and genetic evolution) is required. Second, advantages and disadvantages of various diagnostic methods and their application to diagnostic investigation along with clinical history should be considered. Finally, proper cow-calf management is necessary for prevention and control of the disease.

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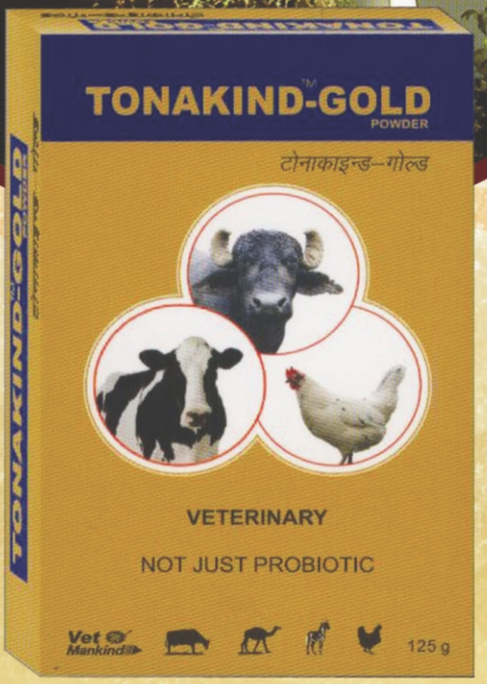

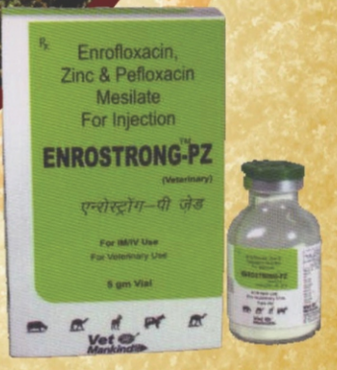

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