Review Article

Neonatal Calf Diarrhea Caused by *Escherichia coli*: Pathogenic Mechanisms and Modern Control Strategies

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Abstract | *Escherichia coli* (*E. coli*) is considered one of the most important bacterial pathogens affecting neonatal calves worldwid*E. coli*bacillosis in calves is primarily associated with enterotoxigenic strains that cause severe diarrhea, dehydration, and high mortality during the first weeks of life, leading to significant economic losses in the cattle industry. The pathogenicity of *E. coli* in calves is linked to virulence factors such as fimbriae (F5, F17, F41), toxins (heat-stable and heat-labile enterotoxins), and the ability to adhere to and colonize the intestinal mucosa. Several risk factors increase calf susceptibility, including poor colostrum intake, unhygienic housing, stress, and co-infections with viruses or protozoa. Clinical signs range from watery diarrhea and weakness to septicemia in severe cases. Diagnosis is based on bacteriological culture, PCR detection of virulence genes, and serotyping. Control strategies focus on good colostrum management, hygiene practices and vaccinate dams to maximize passive immunity. Antibiotics are commonly used; however, the emergence of multi-drug resistant forms of *E. coli* have made practitioners wary and has opened the door to seek alternative therapies, such as probiotics, prebiotics, bacteriophages and plant-derived antimicrobials. In conclusion, *E. coli* infection of calves will be part of veterinary medicine and animal production for a long time. Evidence suggests the best hope of controlling the infection response in calves with *E. coli* is to have a multi-faceted prevention, rapid diagnosis and judicious therapy without the risk of poor prognosis in the future, while reducing calf morbidity and mortality and AMR associated with calf colibacillosis.

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Introduction

Neonatal calf diarrhea (NCD) remains the most frequent and economically important health concern within cattle production systems around the world. Many different pathogens are associated with NCD, but *E. coli* (especially enterotoxigenic *E. coli* (ETEC)) is the most important pathogen associated with morbidity and mortality in the first weeks of life. Calves suffering from diarrheal disease incur direct losses from death, as well as poor growth performance, increased treatment costs and losses from decreased productivity over time (O'Beirne *et al*., 2023). The multifactorial effects exhibited by NCD, with the convergence of pathogens, host immunity, environment and management, means that despite many generations of research, effort and management, *E. coli* infections continue to be a significant challenge to veterinary science and the economy as a whole (Tóthová *et al*., 2024). The pathogenicity of *E. coli* in calves is associated with virulence factors which enable attachment, colonization and toxin production. Fimbrial adhesins, such as F5 (K99), F41, and F17, allow attachment to intestinal epithelial cells, while enterotoxins, such as heat-stable toxin (STa) induce the secretion of fluids and watery diarrhea (Lan *et al*., 2023). This process is highly influential in calves less than one week of age, when the gastrointestinal system and immune system of the calf has not fully matured (Meenakshi *et al*., 2024). Of particular note, calves have also been found to harbor enteropathogenic *E. coli* (EPEC) and Shiga toxin (Stx) producing *E. coli* (STEC), although these strains' role in disease is heterogeneous and inconsistent compared to enterotoxigenic *E. coli* (ETEC) (Bonelli *et al*., 2021). There are several predisposing factors that influence whether the exposure to pathogenic *E. coli* will cause clinical disease. Firstly, inadequate transfer of passive immunity in a calf through the intake of colostrum, which may occur due to its quality, timing and/or volume, will elevate the risk, primarily due to the dependence of calves on passive transfer of maternal antibodies for the first few weeks of life to protect them against disease (Tóthová *et al*., 2024). Another major consideration is environmental factors around the calf, such as hygiene, housing density, and climate stress, that can affect exposure or infective dose and overall calf resilience (O'Beirne *et al*., 2023). Other possible confounding factors are coinfections with other pathogens (for example rotavirus, coronavirus or Cryptosporidium parvum) that can affect severity as well as the complexity of diagnosing (Meenakshi *et al*., 2024). *E. coli* infection presentation can vary; however, the classic presentation is sudden onset watery diarrhea that may evolve quickly to dehydration, electrolyte imbalance and in a very small proportion of serious cases, bacteremia and septicemia (although the mortality rates associated with these untreated conditions is often high). Large surveillance studies in the past few decades give now veracity that the evolving discovery of new strategies for diagnosing *E. coli* infections has transitioned from the traditional bacteriological culture and serotyping, to the utility of newer molecular diagnostic methods; polymerase chain reaction (PCR), and most importantly whole-genome sequencing (WGS) which enables additional characterization of isolates according to their virulence genes and antimicrobial resistance determinants (Gong *et al*., 2023). Management of *E. coli* infection is supportive in therapy, reducing the vomiting and providing electrolytes with oral or IV fluids, normalized acid–base balance and so forth. Antimicrobial therapy may be necessary for systemic cases, however, the rising rise of antimicrobial resistance (AMR) and the associated calf *E. coli* isolates is becoming a larger global concern. Resistance to critically important drug classes due to plasmids and other mobile genetic elements is also extremely worrisome and it highlights the need for good antimicrobial stewardship and alternative preventative approaches (Vischi *et al*., 2023; Lima *et al*., 2024). Prevention still serves as the primary means of control, focusing on colostrum management, hygiene, the vaccination of pregnant cows with fimbrial antigens, and new methods, including probiotics, prebiotics, and bacteriophages.

Pathogenesis and virulence factors

Neonatal calf disease (NCD), which is mostly comprised of *Escherichia coli* (*E. coli*) infections is primarily caused by enterotoxigenic (ETEC) strains, which are the most significant underlying cause of neonatal calf disease. For *E. coli* to cause disease, the organism must possess specific virulence factors, which can be grouped into two pathogenicity groups which include adhesins, as a colonization factor in the intestinal mucosa and enterotoxins, which interferes with fluid homeostasis. The combination of these two pathogenicity factors result in acute diarrhea and dehydration, and in the worst-case outcome, bacteremia and septicemia (Lan *et al*., 2023). Adhesins are part of virulent factors, belonging to fimbriae or pili, that are important for the capacity of *E. coli* to attach to epithelial cells in the small intestine. The most important fimbrial adhesins implicated noticed via negative-cock horse conformation with calf colibacillosis disease include F5 (K99), and F41 and F17. F5 (K99) is the form of ETEC that is most likely to be associated with diarrhea in calves < 1 week of age because of their fimbral attachment to receptor sites on immature enterocytes (Meenakshi *et al*., 2024). The capacity of fimbriae to help ETEC overcome peristalsis and to attach at the mucosal surface of the gut is the first significant milestone in the development of diarrhea caused by ETEC in calves. Once the bacteria have attached, they proliferate in abundance at substantially high rates, forming a dense population of ETEC that can effectively deliver toxins to the animal. Enterotoxins represent the second principle virulence factor of ETEC. Among two human pathogens associated with ETEC (heat-labile toxin and heat-stable toxin), the enterotoxin most relevant in calves is heat-stable enterotoxin (STa). It activates guanylate cyclase receptors of enterocytes - receptors that increase cyclic GMP approximately 10-fold in the cytosol of the cell, further regulating chloride and water secretion into the intestinal lumen. This results in profuse watery diarrhea that can rapidly cause dehydration and metabolic acidosis. Heat-labile toxin (LT) is also mentioned in the case of ETEC isolates from humans, but is less frequently cited as an involved enterotoxin in ETEC calf diarrhea cases. Other strains of ETEC may also produce secreted autotransporter toxins, and outer membrane proteins that could potentially be involved in damaging the intestinal mucosa and inflammation (Lan *et al*., 2023). Other pathogenic types of *E. coli* are implicated in disease conditions in calves. Enteropathogenic *E. coli* (EPEC) strains use the locus of enterocyte effacement (LEE) pathogenicity island, which leads to attaching-and-effacing (A/E) lesions in the gut. The A/E lesion consists of a variety of changes to the enterocytes, including intimate attachment to enterocytes with destruction of microvilli and inhibition of intestinal nutrient absorption ultimately increasing the severity of diarrhea (O'Beirne *et al*., 2023). Shiga toxin-producing *E. coli* (STEC), although a much larger significance to human infection, is found in calves, and its importance in NCD is tentative, but presents a serious zoonotic risk (Vischi *et al*., 2023). In rare instances the consequence of bacterial translocation across an incompetent intestinal barrier can cause systemic invasion by the bacteria into the bloodstream resulting in septicemia; this translocation may be a high-risk factor with colostrum-deprived calves that exhibit low immunoglobulin circulating levels. Systemic translocation of pathogenic bacteria usually associates with high mortality rates in infected calves and urgencies for therapeutic intervention, particularly as previous work has detailed that septicemia among calves is associated with high mortality (Tóthová *et al*., 2024). The new areas in molecular epidemiology and whole-genome sequencing (WGS) have significantly improved the understanding of the genetic diversity of virulence determinants of calf *E. coli*. Across many fecal *E. coli* isolates from past workers, virulence genes can occur with mobile genetic elements like plasmids that allow for horizontal gene transfer between strains, while also limiting downstream control of the *E. coli* strain (Gong *et al*., 2023). These characteristics are essential for the future development of livestock vaccines and targeted therapies. Lastly, it is essential to identify that the pathogenesis of *E. coli* in neonatal calves is a multifactorial process involving fimbrial adhesion and toxin-mediated fluid losses which can systemically distribute toxins to tissue. The underlying severity of the disease is a result of a complex interaction between individual virulence factors and the host susceptibility (i.e., due to the immaturity of the newborn immune system). Future research must place a considerable amount of effort on molecular characterization and immune-mediated responses to improve existing interaction of infection or new interventions.

Epidemiology and risk factors

Neonatal calf diarrhea (NCD), caused by *Escherichia coli* (*E. coli*) is widely recognized as a disease affecting both dairy and beef herds. The epidemiology of colibacillosis in calves is complicated by interactions between virulence of the pathogen, level of exposure of calf immunity, and contributing environments and management factors. Enterotoxigenic *E. coli* (ETEC), especially F5 (K99) and F41 and F17 types, are amongst the most commonly described isolates from diarrheic calves aged under a week (Meenakshi *et al*., 2024). While ETEC is the principle pathogen contributing to NCD, at times, additional pathotypes such as enteropathogenic *E. coli* (EPEC) and Shiga toxin–producing *E. coli* (STEC) are detected and may contribute to disease burden or zoonosis potential (Vischi *et al*., 2023). Epidemiologic surveys in Europe, Asia, and South America indicate that *E. coli* is among the top bacterial agents associated with neonatal diarrhea. However, the prevalence reported for *E. coli* in calves will vary based on diagnostic methodology, the age of calves in the study population and the management protocols at farms (Tóthová *et al*., 2024). A recent study of veal and dairy calves in Italy reported a high prevalence of multidrug-resistant *E. coli* isolates with the authors suggesting antimicrobial resistance (AMR) should be included as part of surveillance efforts involving all agents in veterinary epidemiology (Vischi *et al*., 2023). In lower income countries where hygiene practices and colostrum management may be less optimal, morbidity and mortality can be particularly high, highlighting the role that environmental factors and management have on the disease process (O'Beirne *et al*., 2023). There are many risk factors for *E. coli* diarrhea in calves, and they are not limited to a single area. One of the primary guiding factors is the amount of colostrum consumed, often called the "four Q's": quantity, quality, quickness, and cleanliness. The risk of failure of passive transfer (FPT) of maternal immunoglobulins increases and the calves will be much more susceptible because colostral antibodies are designed to protect neonates from enteric pathogens during their early days of life (Tóthová *et al*., 2024). Poor colostrum management is one of the best predictors of NCD. Environmental cleanliness is also paramount. For example, calves on high densities of stocking, bedding fecal contamination, or poor sanitation in calving pens will be more likely to be exposed to pathogens. Seasonal peaks of NCD often occur in cold, wet months when calves are under environmental stressors that can also reduce immunity (O'Beirne *et al*., 2023). Stress experienced from inadequate nutrition, stressful movement, or abrupt weaning may also pose risks of colibacillosis to calves through immune suppression. Another idea within epidemiology is the idea of co-infections. That is, viruses like bovine rotaviruses and coronaviruses and protozoans like Cryptosporidium parvum are often present in association with *E. coli* in diarrheic calves. There is evidence to suggest that with mixed infections, the severity of the disease is often worse, treatment costs are increased, and making a diagnosis is more impossible (Meenakshi *et al*., 2024). The examples of associations outlined provide evidence of the multifactorial nature of NCD and the importance of diagnostics, in addition to the possible importance of the presence of those pathogens or the consideration of co-infection in the etiology of NCD. Finally, another epidemiological consideration is the emergence of antimicrobial resistance in *E. coli* isolates from calves. Resistance not only complicates therapy, but it could also be a possible source of resistance genes that affect both animal and human health (Lima *et al*., 2024). The recent identification of extended-spectrum β-lactamase (ESBL)-producing *E. coli* isolated from calves is a reminder of the need for approaches based on One Health with continuous surveillance for trends of resistance and the effectiveness of one health initiatives. In conclusion, the epidemiology of *E. coli* (despite its simplicity) in neonatal calves is influenced by pathogen factors, the host, immunity, the environment, and management. Therefore, successful preemptive measures can only be taken if the risk factors can be tackled in a cumulative way through improving management of colostrum, hygiene, stress, and integrated surveillance for AMR.

Clinical signs and diagnosis

Neonatal calf diarrhea (NCD), resulting from *Escherichia coli* (*E. coli*), particularly enterotoxigenic types (ETEC), presents with characteristic clinical signs that vary in degree depending on calf age, vigor and other disease processes occurring at the same time. Calves are affected most severely at age less than 1 week, as calostral antibodies are the main defense during this time (Lima *et al*., 2024) and failure of passive transfer (FPT) puts them at higher risk for severe disease. The clinical signs start typically with watery diarrhea, which can be yellowish to whitish depending on diet and co-infecting agents. Calves become severely dehydrated quickly, with signs including sunken eyes, skin tenting, dry mucous membranes and cold extremities. Systemic illness usually results from dehydrated calves from fluid and electrolyte imbalances, leading to metabolic acidosis and hypovolemic shock (Aghamohammadi *et al*., 2019). Weakness progresses and they do not want to suckle or maintain a standing position with advanced signs of disease. Septicemia may occur when invasive *E. coli* strains invade the intestinal mucosa, resulting in polyarthritis, meningitis or pneumonia that often ends fatally if not treated promptly (Constable *et al*., 2023). Mortality rates can be as high as 50% in untreated calves, highlighting the clinical and economic significance of the disease. Diagnosis of colibacillosis in calves requires a combination of clinical assessment and laboratory confirmation. At the herd level, diarrhea outbreaks in calves under seven days of age with rapid dehydration strongly suggest ETEC involvement (Meenakshi *et al*., 2024). However, because diarrhea in calves is multifactorial, laboratory diagnostics are essential to confirm *E. coli* as the causative agent and to differentiate it from viral (rotavirus, coronavirus) and protozoal (*Cryptosporidium parvum*) infections. Traditional diagnostic methods include bacterial culture and biochemical identification of *E. coli* isolates. Serotyping for fimbrial antigens (e.g., F5, F41) and toxin assays (e.g., heat-stable toxin STa) help confirm pathogenic strains (Shahrour *et al*., 2022). Rapid antigen detection tests, including latex agglutination and ELISA, represent examples of new screening techniques used in the field. Molecular methods have advanced the diagnostics of pathogenic *E. coli*. Using polymerase chain reaction (PCR) assays to identify virulence genes associated with fimbriae and toxins, clinicians can obtain a highly sensitive and specific diagnosis (Meenakshi *et al*., 2024). The emergence of multiplex PCRs and quantitative PCR (qPCR) allows an affordable diagnostic test to identify multiple pathogens at the same time (should be useful in this situation since calf diarrhea is commonly due to co-infection of pathogens) (Vischi *et al*., 2023). Whole-genome sequencing (WGS) and next-generation sequencing (NGS) are now being used in an increasing number of epidemiological studies. These advanced forms of molecular diagnostics allow for strain typing, antimicrobial resistance profiles, and are a reference to find new virulence determinants (Constable *et al*., 2023; Sweeney *et al*., 2019). The development of point-of-care diagnostics, such as portable PCR systems and biosensor assays, are aimed at providing field-based rapid diagnostics and potentially early decision making regarding therapeutics. At this time, while similar technologies may not be widely employed in the overall diagnostic process for veterinary medicine, they may become viable options for potential management of calf colibacillosis in the future (Meenakshi *et al*., 2024).

Treatment and antimicrobial resistance

The treatment for neonatal calf diarrhea (NCD) due to *Escherichia coli* is ultimately supportive, including the responsible use of antimicrobials in severe or systemic cases. Effective management is characterized by expedient treatment of dehydration, electrolyte derangements, and metabolic acidosis, which are the major causes of morbidity and mortality (Constable *et al*., 2023). For calves that have mild to moderate diarrhea, commercial oral rehydration solutions (ORS) with appropriate concentrations of sodium, glucose, and alkalinizing agents are recommended. Severely dehydrated calves or active, recumbent calves would benefit from intravenous fluids (Aghamohammadi *et al*., 2019). Nutritional support should be given as soon as possible to prevent depletion of reserves and maintain energy metabolism. Providing milk or milk replacer as well as an ORS is standard practice. Antimicrobial treatment should only be used in the ill calf exhibiting systemic disease such as fever, sepsis, or evidence of invasive disease. Used antimicrobials include aminopenicillins, cephalosporins, and fluoroquinolones, based on local susceptibility patterns, and all should follow guidance from local veterinarians (Lima *et al*., 2024). Nonetheless, the emergence of multi-drug resistant (MDR) *E. coli* in neonatal calves has become an enormous global concern. Reports of resistance to various classes of antimicrobials such as β-lactams, tetracyclines, aminoglycosides, and sulfonamides, usually associated with plasmids and mobile genetic elements, have been documented (Vischi *et al*., 2023). The identification of extended-spectrum β-Lactamase (ESBL)-producing *E. coli* as an intrinsic gut microbe further delays any therapeutic decisions and this mandates susceptibility testing, and preventing future emergence of resistance to,(likely in the early treatment stages is not possible, highlighted the importance of ability to conduct susceptibility testing, is and by implications, preventing future emergence of resistance, is critical). With the prevalence of antimicrobial resistance (AMR) only increasing, antimicrobial stewardship is paramount to tackling AMR. This involves using antibiotics strictly for confirmed or suspected bacterial infections, using narrow-spectrum antibiotics if possible, following manufacturers recommendations in terms of dosage, and treating the prescribed duration alternatively (Bonelli *et al*., 2021). Although minimize antibiotic usage is important, preventative measures are essential. Maternal vaccines and better management of colostrum supplements will lessen the demand (and utilize of antibiotics) because of fewer disease conditions. Because of the discussions surrounding antibiotic use; alternatives to antibiotics, probiotics, prebiotics and synbiotics used to modulate intestinal microbiota, enhance mucosal immunity, and reduce the severity and duration of diahrrea in calves are becoming increasing popular (Meenakshi *et al*., 2024). Bacteriophage therapy, still experimental in most areas, provides specific, targeted treatment of pathogenic *E. coli* and does not contribute to AMR. Phytochemicals and plant-based compounds have shown antimicrobial activity in vitro, and may be likely to be used preventively in addition to other treatments. Monitoring and surveillance of trends in AMR in *E. coli* populations is an important part of herd-level management, and more broadly, public health. Whole-genome sequencing (WGS) and molecular methods for detection of resistance genes give directions for how AMR is spreading, and the ability to guide evidence-based strategies (Gong *et al*., 2023). Multiple therapy programs that combine supportive care, antimicrobial stewardship, and preventatively supported therapies will be necessary to lessen not only clinical disease but also the development of resistant bacterial populations.

Prevention and control strategies

Ideal prevention and control of *Escherichia coli* (*E. coli*) infections in neonatal calves will take an integrated, multifaceted approach, considering the pathogens they are exposed to, the immunity of the host and the environment in which they live. This is particularly beneficial when we consider that ETEC, and a wide variety of pathogenic *E. coli*, use the flaws of the neonatal immune system against our hosts, and we should attempt to improve host resistance to *E. coli* and to decrease the bacterial challenge to the neonatal calf (Tóthová *et al*., 2024). Colostrum management will be a leading aspect of a preventative strategy. Sufficient passive transfer of maternal antibodies will provide valuable protection against enteric pathogens that the calf is exposed to in their first few weeks of life. Newborn calves should consume a good quality colostrum, free from bacterial contamination, within the first 2-4 h of birth. If the calf consumes as a total at least 10% of their body weight in colostrum during the first 24 h of life, expect that a sufficient transfer of immunity has taken place from the maternal colostrum (O'Beirne *et al*., 2023). Quality control of colostrum using a colostrometer or Brix refractometer will ensure adequate immunoglobulin levels and clean collection and storage will prevent bacterial growth. Vaccination of pregnant cows against ETEC fimbrial antigens (termed F5/K99 and F41) is another preventive process. Vaccinated dams will transfer specific antibodies in colostrum, which substantially limits both occurrence and severity of diarrhea in their calves (Lan *et al*., 2023). Vaccination against other enteric pathogens, e.g., rotavirus, coronavirus and Clostridium perfringens, can be applied in conjunction for protection against multifactorial causes of neonatal diarrhea. Hygiene and environmental management are also vital to reduce pathogen exposure. Regular washing and disinfecting of calving pens, housing neonates separately, keeping pens dry and calm to reduce stress and providing adequate air flow will minimize bacterial load. Bedding should be kept dry and replaced often, and cleaning and disinfecting calf feeders and waterers can reduce cross contamination (Meenakshi *et al*., 2024). Environmental monitoring of fecal contamination has the potential to identify areas of high risk and facilitate targeted interventions, while alternative options are being recognized as complementary means for the prevention of fecal contamination. Probiotics and prebiotics affect gut microbiata, enhance mucosal immunity, and inhibit the colonisation of pathogenic *Escherichia coli* from the intestinal epithelium via competitive exclusion (Lima *et al*., 2024). Bacteriophage therapy is still largely experimental, but provides a targeted approach to specific pathogenic strains via bacteriophage incorporation without a likelihood of antimicrobial resistance. Also, plant-derived compounds and immunomodulators were effective in reducing diarrhea severity in calves on controlled trials (Vischi *et al*., 2023). Surveillance and early detection in diseases like *E. coli* are critical components of prevention. Routine health examinations, surveillance for early signs of diarrhea, and rapid diagnostic tests provide opportunities for efficient intervention that can impact disease and death rates. In addition, antimicrobial stewardship programs, particularly with regard to monitoring resistance profiles, are vital to halt the emergence and spread of multidrug-resistant *E. coli* strains in herds (Bonelli *et al*., 2021). Finally, the most comprehensive workplace health measures involve an integrated herd health program that follows best practices for managing colostrum intake, vaccination protocols, biosecurity in the environment, optimizing nutrition, and regular veterinary visits. Educating farm personnel about evidence-based practices, early signs of illness and assemblages for treatment allows for continued herd health and productivity.

Conclusion

Neonatal calf diarrhea due to *Escherichia coli* continues to be a serious health and economic issue. Proper management of neonatal calf diarrhea relies on providing adequate amounts of colostrum, hygiene, vaccination, and a judicious approach to antibiotic use. The improved use of molecular diagnostic services, access to alternative therapies, and herd health programs can help to lessen incidence, severity and antibiotic resistance, leading to healthier calves and more productive results.

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Novelty Statement

This study aims to integrate molecular principles with traditional methods to better understand Escherichia coli infection in young calves, provide innovative non-antimicrobial alternatives to control antimicrobial resistance, and provide better management strategies to improve calf health.

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Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

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