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Pathogen-driven pregnancy loss in dairy cattle: An overview of diagnostic advances and innovations

Nina Herlina^{1,2} , Safika³ , Ekowati Handharyani⁴  and Agus Setiyono^{4*} 

¹Animal Biomedical Study Program, IPB Postgraduate School, School of Veterinary Medicine and Biomedicine, IPB University, Bogor, Indonesia

²Center for Applied Zoology, Research Organization for Life Sciences, National Research and Innovation Agency (BRIN), Cibinong Science Center, Bogor, Indonesia

³Division of Microbiology, School of Veterinary Medicine and Biomedicine, IPB University, Bogor, Indonesia

⁴Division of Pathology, School of Veterinary Medicine and Biomedicine, IPB University, Bogor, Indonesia

ABSTRACT

Pregnancy failure or abortion in dairy cattle is a major reproductive concern that affects animal welfare and the dairy industry's economic stability. Pathogens are a key factor contributing to this issue. Accurate diagnosis is essential for effective management and preventive measures. This review discusses various pathogens responsible for abortion in dairy cattle, including bacteria, viruses, and protozoa. Conventional diagnostic methods, which are important, may not always comprehensively detect all pathogens involved in abortion. This study focuses on current advances in diagnostic technology, particularly molecular approaches such as polymerase chain reaction and enzyme-linked immunosorbent assays, as well as the promise of metagenomic techniques for increased accuracy. By integrating recent research findings, this review emphasizes the significance of these advancements in enhancing disease management, prevention measures, and overall herd health.

Keywords: Abortion, Dairy cattle, Diagnostic, Metagenomic, Molecular.

Introduction

Pregnancy loss in dairy cattle, which occurs as abortions between 42 and 260 days of gestation, poses a major challenge for the dairy industry (Mee, 2020). Up to 20% of pregnancies are affected by this problem, which causes significant financial losses for the dairy industry (Albuja *et al.*, 2019). Abortions in dairy cows result in higher expenses for reproduction, veterinary services, and feed while adversely impacting milk yield, fertility, and overall health, leading to increased culling and turnover rates (Keshavarzi *et al.*, 2020). Abortion rates typically range from 1% to 2%. Finding the root causes requires careful consideration if this rate rises above 2%–5%. To investigate causes, potential remedies, and preventive actions, more assessment is necessary (Van Loo *et al.*, 2024). According to Serrano-Martínez *et al.* (2019), abortions may be caused by infectious agents, factors of unknown origin, or noninfectious causes. Abortion in cattle is caused by noninfectious conditions, such as hormonal imbalances, metabolic abnormalities, heat stress, physical damage, and malnutrition. These factors may interfere with a healthy pregnancy and result in fetal death (Khan *et al.*, 2023). Other factors linked to abortion are the consumption

of toxic plants, exposure to mycotoxins, and hormonal fluctuations due to progesterone deficiencies or the use of glucocorticoids, oxytocin, estrogen, and prostaglandins. Nutritional insufficiencies, genetic factors, chromosomal disorders, umbilical torsion, and twin pregnancies can also contribute (Yadav *et al.*, 2021). Toxins from certain plants, such as *Astragalus* species and *Ponderosa* pine needles, along with estrogenic mycotoxins can further lead to abortion incidents (Buroni *et al.*, 2020). Various infectious agents, including bacteria, viruses, protozoa, and fungi, have been implicated in bovine abortion.

Many factors, particularly infectious agents, contribute to pregnancy loss and perinatal deaths in cattle. Girmay *et al.* (2024) found that the seroprevalence of bovine viral diarrhea virus (BVDV) and BHV1 was higher in dairy cows with reproductive problems in Ethiopia than in *Neospora caninum* and *Chlamydia burnetii*. A number of agents associated with cattle reproductive failure, including *Salmonella enterica*, *Chlamydia abortus*, *Listeria monocytogenes*, *Brucella* species, *Leptospira* species, *C. burnetii*, *C. abortus*, *S. enterica*, *Campylobacter* species, and *Yersinia enterocolitica*, pose zoonotic risks (Ebani, 2022). These pathogens

*Corresponding Author: Agus Setiyono. Division of Pathology, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia. Email: agusse@apps.ipb.ac.id

are therefore crucial to public health and animal husbandry. Using the One Health concept, cooperation between veterinary and medical specialists is crucial for successfully reducing illnesses linked to fertility (Sevik, 2025). However, despite improvements in diagnostic methods, the identification of the causes of reproductive losses has not surpassed 50% accuracy and remains stagnant over time. The dairy industry faces economic losses due to expected calf deaths and resulting delays in milk production, which are vital to farm profitability.

Identifying these pathogens quickly and accurately is essential for effective management and control. Laboratories performing abortion diagnostic tests should include standard assessments for the main infectious diseases linked to abortion. Prioritizing the most prevalent abortifacient drugs is crucial for efficient disease management and control, even when financial limitations may restrict the range of tests. Traditional diagnostic techniques are crucial, but they usually overlook different causal agents, emphasizing the need for improvements in diagnostic technologies.

Pathogens Involved in Bovine Abortion

Worldwide studies indicate that *N. caninum* (22.2%) is the most common pathogen. Meanwhile, *C. burnetii* was found at the lowest percentage, at 9.5%. The percentages of opportunistic bacteria and the *Chlamydiaceae* family were in the middle, with 10.9% and 21.4%, respectively (Hecker et al., 2023). Vidal et al. (2017a,b) investigated the prevalence of zoonotic infections in cases of bovine abortion in Switzerland. According to the findings, *Coxiella burnetii* was found in 15.9% of cases, *C. abortus* in 38.5%, and *Leptospira* species in 21.4%. The study revealed that diagnostic tools, including real-time polymerase chain reaction (PCR) and histological examinations, should be utilized to enhance the specific identification of bovine abortion instances and accurately determine the prevalence of these zoonotic infections. Pathogens such as bovine herpesvirus type 1 (BoHV-1) and bovine viral diarrhea (BVD) occur at rates of approximately 5%. Intermittently isolated or opportunistic pathogens, such as *Escherichia coli*, *Salmonella* spp., *Staphylococcus* spp., *Pasteurella* spp., *Pseudomonas* spp., *Trueperella pyogenes*, *Streptococcus* spp., *Bacillus licheniformis*, *Enterobacter* spp., *Pajaroellobacter abortibovis*, *Klebsiella* spp., *Acinetobacter* spp., *Histophilus somni*, *Bordetella* spp., *Actinomyces* spp., *Yersinia paratuberculosis*, *Aeromonas* spp., and *Cardiobacterium* spp., were observed in 21.4% of cases (Hecker et al., 2023). Table 1 shows the list of pathogenic microorganisms causing abortion in dairy cattle in the past 5 years (2019–2024).

Neospora caninum

Dubey et al. (1988) reported that *N. caninum* is a parasitic organism from the Apicomplexa family that primarily affects dogs but also a variety of animals,

especially livestock. The parasite has a life cycle involving definitive hosts, mainly dogs and other canids, and intermediate hosts such as cattle. Dog waste can contain oocysts and can contaminate food, water, and the surroundings. Cattle become infected by consuming these oocysts. Vertical transmission from mother to fetus is also crucial, as this facilitates the spread of infection within herds (Dubey, 2003). The protozoan parasite *N. caninum* plays an important role in abortion in cattle. Infection can occur through horizontal transmission via the ingestion of oocysts expelled by canids or vertically, from an infected mother to her fetus, potentially leading to abortion, stillbirth, or the birth of offspring that are subclinically infected (Gluga et al., 2025). In dairy cattle, abortion is the main symptom, often occurring in the second trimester. Infected cows may also experience stillbirths, premature births, and weak calves (Buxton et al., 2002). *N. caninum* is the leading abortifacient agent in both dairy and beef cattle. A 2023 study in Egypt highlighted the substantial role of *N. caninum* in causing abortions among dairy cattle, emphasizing the parasite's contribution to economic losses in the livestock industry (Selim et al., 2023). Infected cows may not show symptoms, but their fetuses can be infected and may experience paralysis or sensory deficits. Diagnosing *N. caninum* in dairy cattle employs various methods. PCR as a molecular approach in identified DNA of *N. caninum* in various tissues and fluids provides fast and precise identification (Buxton et al., 2002). Moreover, histopathological analysis of aborted fetuses can detect specific lesions, including nonsuppurative encephalitis and myocarditis, and help identify the parasite in tissues (Dubey and Lindsay, 1996). Various serological techniques, such as the indirect fluorescent antibody test (IFAT), enzyme-linked immunosorbent assays (ELISA), immunoblotting, and direct agglutination tests, are available for detecting specific antibodies against *N. caninum* in cattle (Paré et al., 1995; Dubey and Schares, 2006; Selim et al., 2023). Udonsom et al. (2024) evaluated the effectiveness of three *N. caninum* proteins as biomarkers for immune-based diagnosis for detecting IgG antibodies against *N. caninum* compared with the IFAT as a reference method for identifying *N. caninum* antibodies. The present study emphasizes NcSAG1 as a valuable antigen marker, whereas NcPrx2 and NcMIC4 show promise for immunodiagnostic applications in detecting *N. caninum* infections in field collections.

Chlamydia abortus

Chlamydia abortus is an intracellular bacterium that leads to significant abortion rates in dairy cattle, resulting in major economic losses for the dairy sector (Longbottom and Coulter, 2003). This pathogen can also infect humans, posing health risks. It is associated with pregnancy losses in cattle, particularly in Argentina. *Chlamydia abortus* is primarily transmitted through the ingesting or inhalation of contaminated materials

Table 1. List of pathogens causing abortion in dairy cattle in the past 5 years (2019–2024).

Country	Pathogen	Detection method	Abortion stage	References
Turkey	Chlamydiae family	qPCR	2nd–3rd trimester	Açici <i>et al.</i> (2019)
Argentina	<i>Neospora caninum</i> , <i>Campylobacter fetus</i> sp., <i>Leptospira</i> spp., <i>Brucella abortus</i> , BVDV and Bovine herpes virus	Histology, bacterial and viral isolation, direct fluorescent antibody test, fetal serology, immunohistochemistry, and PCR	1st–3rd trimester	Morrell <i>et al.</i> (2019)
Peru	<i>N. caninum</i>	PCR, ELISA, histopathology	1st–3rd trimester	Serrano-Martinez <i>et al.</i> (2019)
Ecuador	<i>N. caninum</i> , <i>Brucella</i> , and <i>C.</i> <i>burnetii</i>	ELISA	NA	Changoluisa <i>et al.</i> (2019)
Belgium	<i>Leptospira</i> spp.	Histopathology and real-time PCR	1st–3rd trimester	Grégoire <i>et al.</i> (2020)
China	<i>Brucella</i> spp	ELISA	NA	Zhang <i>et al.</i> (2020)
Iran	<i>N. caninum</i>	PCR	1st–3rd trimester	Salehi <i>et al.</i> (2021)
Indonesia	<i>Chlamydia</i> spp., BVD, and <i>N.</i> <i>caninum</i>	ELISA	NA	Subekti <i>et al.</i> (2021)
India	Bovine alphaherpesvirus-1 (BoHV-1), BVDV, <i>N. caninum</i> , <i>B. abortus</i> , <i>Coxiella burnetii</i> , <i>Leptospira</i> , and <i>Listeria</i> <i>monocytogenes</i>	PCR, ELISA	2nd–3rd trimester	Sarangi <i>et al.</i> (2021)
Denmark	<i>Escherichia coli</i> , <i>Streptococcus</i> <i>pluranimalium</i> , and <i>S. equinus</i>	The 16S rDNA amplicon sequencing–guided FISH approach	NA	Wolf-Jäckel <i>et al.</i> (2021)
Brazil	<i>C. burnetii</i>	qPCR	NA	Mioni <i>et al.</i> (2022)
Brazil	<i>C. burnetii</i>	Histopathology, PCR	NA	Silva da Costa <i>et al.</i> (2022)
Ethiopia	<i>B. abortus</i> , <i>Coxiella burnetii</i> , and <i>Leptospira</i> Hardjo	ELISA	NA	Robi <i>et al.</i> (2023)
Egypt	<i>N. caninum</i>	ELISA	NA	Selim <i>et al.</i> (2023)
Botswana	<i>Brucella</i> spp., <i>Coxiella burnetii</i> , <i>Leptospira</i> spp., <i>L. monocytogenes</i>	PCR	NA	Modise <i>et al.</i> (2023)
Korea	BVDV, <i>Coxiella burnetii</i> , <i>Leptospira</i> spp., <i>L.</i> <i>monocytogenes</i> , and <i>N. caninum</i>	ELISA	NA	Kim <i>et al.</i> (2024)
China	<i>C. burnetii</i>	PCR	NA	Liu <i>et al.</i> (2024)
Italy	<i>Chlamydia abortus</i> , <i>Coxiella</i> <i>burnetii</i> , <i>Salmonella</i> spp, <i>Toxoplasma gondii</i> , and <i>N.</i> <i>caninum</i>	qPCR	NA	Santori <i>et al.</i> (2024)

such as aborted fetuses, placental tissues, and vaginal secretions. It may also be transferred vertically from the mother to the fetus (Wheelhouse and Longbottom, 2015). Dairy cattle, along with other ruminants such as sheep and goats, are key reservoirs for this bacterium. Infected animals shed the bacteria, contaminating their surroundings and spreading the infection among herds (Borel *et al.*, 2018). The most common clinical symptom in dairy cattle is abortion, which usually

occurs in the latter trimester. Other reproductive issues include stillbirth, weak calves, and infertility (Longbottom and Coulter, 2003). Infected cows might show symptoms of systemic illness such as fever and lethargy, although many cases can be asymptomatic, complicating detection and management (Wheelhouse and Longbottom, 2015). Diagnosis of *C. abortus* in dairy cattle uses various methods. Serological tests, such as the complement fixation test (CFT) and ELISA,

help identify antibodies against bacteria, revealing both acute and chronic infections (Sachse *et al.*, 2009). Molecular techniques such as PCR frequently help to identify *C. abortus* DNA in samples such as placental tissues, vaginal swabs, and aborted fetuses, providing a quick and precise diagnostic method (Pantchev *et al.*, 2009; Sachse *et al.*, 2009). Although it is feasible to isolate *C. abortus* from clinical samples, this is difficult because of the bacterium's intracellular nature and the need for specialized lab facilities (Borel *et al.*, 2018). Pinto *et al.* (2025) identified 2.2% positivity for the Chlamydiaceae family in pregnancy loss of ruminants using real-time PCR (qPCR).

Brucella abortus

Brucella abortus is a highly contagious bacteria classified as a Gram-negative coccobacillus that results in a zoonotic disease, brucellosis, which affects animals and public health (Wang *et al.*, 2024). The disease spreads substantially through direct contact with infected creatures or their concealment, including abandoned fetuses, placental accouterments, and uterine fluids. Contaminated feed, water, and equipment can also transmit the disease (Khurana *et al.*, 2021). Wild animals such as deer and bison act as reservoirs, making their control challenging in cattle herds (Moreno, 2014). Common signs of dairy cattle include late-term abortions, retained placenta, and metritis (Narnaware *et al.*, 2017). Herds may experience abortion storms (Zhou *et al.*, 2021). Infected cows often exhibit diminished milk production, whereas bulls may show signs of orchitis and arthritis (Radostits *et al.*, 2007). The diagnosis of brucellosis involves various methods. Serological tests, such as the Rose Bengal test, CFT, and ELISA, are frequently used to identify antibodies against *Brucella* (Rahman *et al.*, 2024). The gold standard for diagnosis remains bacteriological methods, which isolate *Brucella* from milk, blood, or tissues (OIE, 2009). Additionally, molecular techniques such as PCR allow for quick and precise detection of *Brucella* DNA in clinical samples (Islam *et al.*, 2025). Rahimoon *et al.* (2024) highlighted that molecular diagnostics outperform traditional methods because they pose no risk to laboratory personnel since DNA is noninfectious. In addition, they offer greater reliability and adaptability.

Coxiella burnetii

Coxiella burnetii is a bacteria that causes Q fever. It is a Gram-negative intracellular organism that affects various animals, especially dairy cattle. This pathogen poses serious health threats to livestock and humans, significantly affecting the dairy industry (Celina and Cerny, 2022). *Coxiella burnetii* is tough, able to survive in extreme conditions and survive in the environment for a long time. The main ways it spreads are through inhaling contaminated aerosols, direct contact with infected animals or their fluids (such as placental tissues, birth fluids, and milk), and consumption of contaminated food or water (Clark and Magalhaes,

2018). Dairy cattle and other ruminants, including sheep and goats, act as major reservoirs. Infected animals can discharge bacteria in their milk, feces, urine, and birthing materials, thereby contributing to environmental contamination and further transmission (Celina and Cerny, 2022). In dairy cattle, *C. burnetii* often leads to reproductive issues such as abortions, stillbirths, weak calves, and infertility, primarily in the last trimester of pregnancy. Although many infected cattle show no symptoms, some may present with fever, lethargy, and mastitis, which can reduce milk production (Gisbert *et al.*, 2024). Several methods have been used to diagnose *Coxiella burnetii* infection in dairy cattle (Sahu *et al.*, 2020). Antibody detection for *C. burnetii* is usually performed using serological tests, such as the CFT and ELISA, in which acute and chronic infections are identified. Molecular techniques, such as PCR, which detects *C. burnetii* DNA in milk, placental tissue, and feces, offer a rapid and specific diagnostic approach (Lyoo *et al.*, 2017; Saegerman *et al.*, 2022). Although *C. burnetii* can be isolated from clinical samples, isolation is rarely accomplished due to the bacterium's intracellular characteristics and the need for specialized labs to grow this fastidious organism (Francis *et al.*, 2020).

Trueperella pyogenes

Trueperella pyogenes is a Gram-positive, facultatively anaerobic bacteria that commonly infects livestock, especially cattle. It is responsible for various pus-producing illnesses. This bacteria produces multiple virulence factors, including pyolysin (a hemolysin), neuraminidase, and various proteases, which increase its ability to cause disease (Wen *et al.*, 2025). It adheres to and invades host tissues, releasing toxins and enzymes that harm host cells and tissues, causing inflammation and pus production (Rzewuska *et al.*, 2019). *Trueperella pyogenes* can induce sporadic abortions at any stage of pregnancy and is often isolated from the placenta and abomasal contents. The fetus frequently lacks identifiable abnormalities, but the placenta may exhibit diffuse inflammation. Currently, there is no effective vaccine for therapy. *Trueperella pyogenes* can be diagnosed using a variety of ways. Blood agar is used in traditional culture procedures to isolate and identify bacteria in clinical samples, such as pus or tissue biopsies. Molecular technologies such as PCR enable speedy and exact detection (Zhang *et al.*, 2015). Furthermore, histopathology examination of tissue samples may reveal the characteristic shape of bacteria as well as any related tissue damage. Recent findings by Wente *et al.* (2024) used MALDI-TOF to identify 151 *T. pyogenes* isolates from 16 herds and described them through RAPD-PCR typing.

Bovine herpesvirus type 1

BoHV-1 is a prominent virus that causes bovine rhinotracheitis (IBR), a contagious disease that has a considerable impact on the cattle industry (Biswas *et al.*, 2013). BoHV-1, an encapsulated double-

stranded DNA virus, belongs to the Herpesviridae and Alphaherpesvirinae subfamily. There are several subtypes, including BoHV-1.1, which is linked to respiratory and reproductive issues, and BoHV-1.2, which is associated with genital infections (Weiqiang *et al.*, 2022). The virus spreads mostly through respiratory secretions, direct contact, and contaminated objects, and can also be transmitted through sexual contact (Brock *et al.*, 2020). BoHV-1 can be latent in the sensory ganglia before reactivating and shedding during stress or immune suppression (Dai *et al.*, 2022). The infection can take many forms, depending on the affected organ systems, age, and immunity of the cattle. Symptoms of the respiratory variant known as IBR include high fever, nasal discharge, conjunctivitis, trouble breathing, and substantial upper respiratory inflammation, which can be worsened by subsequent bacterial infections. In cows, the reproductive form can cause abortion, infertility, and vulvovaginitis, whereas bulls can develop balanoposthitis. The ocular variant manifests as conjunctivitis and keratitis, frequently with respiratory symptoms (Brock *et al.*, 2020). BoHV-1 infection in young calves can result in severe disease with significant mortality rates (Lemaire *et al.*, 2000). Antibodies against BoHV-1 can be detected using procedures like ELISA and virus neutralization tests (VNT), indicating a previous or present infection. PCR is widely used to detect BoHV-1 DNA in nasal swabs, blood, and tissue samples, enabling a rapid and accurate diagnostic alternative. Although virus culture using clinical samples can prove BoHV-1, it is time-consuming and requires specialized equipment. Furthermore, identifying viral antigens in tissue samples may be helpful in the diagnosis of abortion cases (Brock *et al.*, 2020).

Bovine viral diarrhea

Bovine viral diarrhea (BVD) is a serious viral disease affecting cattle worldwide. The disease is caused by a virus from the genus *Pestivirus* belonging to the Flaviviridae family. This disease has significant economic consequences for the dairy and meat industries because it affects animal health, reproduction, and productivity (Tura *et al.*, 2025). BVDV has two biotypes: cytopathic (cp) and non-cp, as well as two primary genotypes: BVDV-1 and BVDV-2, each with many subgenotypes (Ridpath *et al.*, 2010). The virus spreads through direct contact with infected animals or contaminated objects (such as feed, water, and equipment). Vertical transmission from cow to fetus is also prevalent, resulting in persistently infected (PI) calves if the fetus becomes infected during early gestation (Smith *et al.*, 2020). PI animals are the main reservoirs of the virus, shedding enormous amounts of it throughout their lifetimes and spreading the infection within herds (Newcomer and Givens, 2016). BVD can manifest in a variety of clinical forms, ranging from asymptomatic to severe disease, depending on the virus strain, host immunological response, and presence of

additional infections. Acute BVD symptoms include fever, diarrhea, respiratory difficulties, decreased milk production, and immunosuppression, which can develop into secondary infections (Baker, 2021). Mucosal illness occurs in PI animals when they are superinfected with a cp strain, resulting in severe gastrointestinal ulcerations, chronic diarrhea, weight loss, and often death (Givens, 2020). BVD also causes reproductive problems, such as abortion, congenital abnormalities, stillbirths, and weak calves, and infected fetuses may suffer deformities, such as cerebellar hypoplasia (Newcomer *et al.*, 2017). Calves infected in utero between 45 and 125 days of gestation become PI animals, acting as lifelong carriers who shed the virus (Grooms, 2018). There are numerous ways for diagnosing BVD. Serological techniques, such as ELISA and VNT, are used to detect antibodies against BVDV, indicating past or current infection (Lanyon *et al.*, 2014). Molecular techniques such as PCR and RT-PCR are commonly employed to detect BVDV RNA in blood, tissue, or milk samples, allowing for a quick and accurate diagnosis (Meyers and Thiel, 2020). Virus isolation, which involves growing the virus from clinical samples in cell culture, can confirm BVDV infection, but this method is time-consuming and requires special equipment (Walz *et al.*, 2020). Furthermore, ear notch testing is a standard method for identifying PI animals by examining ear notch samples using PCR or antigen-capture ELISA (Ridpath *et al.*, 2012).

Diagnostic Advances and Innovations

Traditional diagnostic methods

Traditional approaches include culture-based procedures and serological assays. Although these methods are useful for detecting specific diseases, they frequently lack the sensitivity and speed required for proper diagnosis. This deficiency can lead to disease underreporting and mismanagement, compromising herd health and productivity.

Molecular diagnostic techniques

The development of molecular diagnostics has transformed pathogen detection. Prior to 2010, most investigations used culture-based methods. Since then, molecular techniques have been used to identify previously unknown bacteria associated with the condition (Galvão *et al.*, 2019). Molecular diagnostics uses molecular biology techniques, especially those involving DNA, RNA, or proteins, to identify specific genes, mutations, or infections in samples. Molecular diagnostics have transformed the detection of infectious agents in dairy cattle (Modise-Tlotleng *et al.*, 2024). These approaches detect low-abundance pathogens with high sensitivity and specificity and provide rapid results, allowing for early intervention. PCR-based assays have been developed to detect a variety of bacterial and viral infections, including *E. coli*, *Salmonella* spp., *T. pyogenes*, bovine herpesvirus 1, and BVDV (Goecke *et al.*, 2021). Wolf-Jäckel *et al.*

(2021) studied abortifacients from 162 aborted or stillborn bovine fetuses and placentas in Denmark using real-time quantitative PCR to detect *Chlamydia* spp. and chlamydia-like organisms. All 162 cases were negative for *Chlamydiaceae* using PCR. Nine instances were positive for *Chlamydiales* by PCR, with DNA found solely in the placenta.

Metagenomic approaches

Over the last two decades, high-throughput DNA sequencing (next-generation sequencing) has rapidly advanced, with new technologies being marketed (Slatko *et al.*, 2018). Metagenomic next-generation sequencing is a revolutionary method for diagnosing infectious diseases. The method uses unbiased high-throughput sequencing to identify and analyze microbial genomes (Zhao *et al.*, 2024). Metagenomics represents a tremendous advancement in diagnostic ability. Bazzazan *et al.* (2025) used metagenomics as an effective technique for studying the uterine microbiome in dairy cattle. This technique enables the simultaneous detection of many pathogens in a single sample, resulting in a thorough understanding of the microbial population. Metagenomic research has identified novel pathogens related to abortion in dairy cattle, including *Fusobacterium necrophorum* and *Porphyromonas levii* (Çömlekcioglu *et al.*, 2024). Metagenomics provides a complete overview of pathogen diversity and abundance by sequencing the DNA of microbial communities in the reproductive tract. This method can distinguish between culturable and nonculturable species, providing a more detailed understanding of the reproductive microbiome and its role in abortion.

Metagenomic techniques provide a thorough examination of the microbial community in the reproductive tract, allowing the identification of possible infections that may lead to abortion (Ong *et al.*, 2021). These sophisticated approaches help us comprehend the complicated interactions between the microbiome and the host, offering light on the etiology of abortifacient agents (Juli *et al.*, 2024). Furthermore, the capacity to detect the virus at an early stage allows for timely intervention, thus minimizing the spread of infection across the herd (Periyasamy *et al.*, 2024). Metagenomic approaches to abortion in dairy cattle can be used using a variety of biological samples, including fetal tissues, placental tissues, and maternal blood (Vidal *et al.*, 2017a,b). By analyzing these samples, researchers can identify potential pathogens and gain a better understanding of the dysbiosis (microbial imbalance) associated with abortion (Appiah *et al.*, 2020).

Metagenomics, which includes whole-genome sequencing or 16S/18S/ITS rRNA gene sequencing, has been shown to be effective in detecting bovine abortion cases. Recent research has shown that metagenomic 16S rDNA sequencing techniques can effectively detect bacterial communities in aborted fetal tissues and placental samples (Wolf-Jäckel *et al.*, 2021). For

example, a study evaluating placental tissues from aborted fetuses using shotgun metagenomics revealed a varied spectrum of bacterial species, including the existences of viral, bacterial, and fungal species that were previously undetectable using conventional approaches (Vidal *et al.*, 2017a,b). Metagenomics has revealed previously unknown pathogens, such as *T. pyogenes*, *C. abortus*, and *Coxiella burnetii*, that focused on PCR approaches had been missed. Another study used long-read sequencing technologies (PacBio, Oxford Nanopore) to enhance genome assembly and taxonomy classification of complicated microbial populations. This indicates the presence of several coinfecting organisms in abortion cases, emphasizing the complexity of microbial interactions (Akter *et al.*, 2021). More precise diagnosis, more focused therapies, and more successful biosecurity measures are all possible with improved pathogen detection (Periyasamy *et al.*, 2024). Table 2 shows a comparison of the features of different diagnostic techniques for identified pathogens of aborted cattle.

Metagenomic techniques can be applied to management procedures, such as reproductive health monitoring and breeding plans. Many studies have indicated that microbial dysbiosis in the reproductive tract may be associated with decreased fertility (Gupta *et al.*, 2024). Adnane and Chapwanya (2024) highlighted the potential of metagenomics to improve our understanding of microbial communities that regulate cattle reproductive health and to guide breeding strategies targeting increasing fertility and decreasing abortion rates.

Genomic approaches

Genome discovery has been one of the most significant developments in the improvement of tools for the genetic enhancement of cattle in recent decades (Gutierrez-Reinoso *et al.*, 2021). Productivity and genetic scanning of genes related to abortion as an effort to prevent pregnancy loss in dairy cattle was reported by Sigdel *et al.* (2021). The study presented the findings of genetic factors linked with abortion in 58,000 Holstein cows in the United States, which included nulliparous, primiparous, and multiparous animals. Seven genomic areas were identified as being implicated in abortion in both heifers and lactating cows: BTA2, BTA10, BTA14, BTA16, BTA21, BTA24, and BTA29. These areas regulate genes involved in pregnancy maintenance and fetal growth, including CHST14, IGF1R, IGF2, PSEN2, SLC2A5, and WNT4. Key scientific terminology and mechanisms related to pregnancy loss include calcium signaling, cell-cell adhesion, cellular proliferation, fetal development, immunity, membrane permeability, and steroid metabolism. The work highlights unique ways to enhance pregnancy maintenance in dairy cows through marker-assisted breeding, leading to a better knowledge of the genetic and biochemical causes of pregnancy loss. Suarez *et al.* (2024) used a genome-wide

Table 2. Comparison of the features of different diagnostic techniques for identifying pathogens in aborted cattle.

Diagnostic methods	Characteristics	Sensitivity	Specificity	Accuracy	Advantages	Limitations	Sampling technique and timing
Culture-based methods	Isolation of bacteria/fungi via growth on selective media	Moderate	High	Moderate	Identifies live organisms	Time-consuming, not suitable for fastidious or unculturable pathogens	Required fresh samples and long incubation times
Serology (ELISA, IFAT)	Detection of pathogen-specific antibodies	Variable (depends on pathogen and timing)	High	Moderate	Fast, cost-effective	Cannot differentiate past and current infections	Blood samples require the host immune response
PCR (conventional/real-time)	Amplification of specific pathogen DNA/RNA	High	High	High	Rapid, highly specific	Prior knowledge of the target pathogen	Tissue, blood, or fetal fluids; sensitive to contamination
Metagenomics (Shotgun, 16S/18S rRNA sequencing)	High-throughput sequencing of total genetic material	Very High	Very High	Very High	Detects known and novel pathogens, culture-independent, comprehensive microbial profiling	Expensive, requires bioinformatics expertise	Any biological sample (tissue, fluids, placenta), real-time or retrospective analysis

association analysis to identify spontaneous abortion in artificially inseminated heifers and discovered that 216 loci and 413 positional candidate genes were linked to spontaneous abortion. Identification of loci associated with spontaneous abortion in artificial insemination-bred heifers may be exploited to reduce fetal loss via genomic selection. Improving the reproductive health of dairy cattle can be significantly improved by incorporating cutting-edge diagnostic methods into standard herd management procedures (Gupta *et al.*, 2024).

Rapid searches for parent-progeny links and identical genotypes among all possible genotypes can significantly reduce processing time. Sequence data are used to identify more informative single-nucleotide polymorphisms and incorporate existing ones. Sequencing-based genotyping can enhance marker selection flexibility. The genetic selection program for dairy cattle has grown significantly both domestically and internationally. More than 50 qualities were evaluated. Recently added metrics included feed efficiency, heifer and cow livability, age at first calving, six health characteristics, and gestation length. To better depict the economic value of selecting candidates, work is being done to establish hoof health evaluations (Wiggans and Carrillo, 2022). Future research should focus on improving these diagnostic tools, lowering

costs, and making them more accessible for regular diagnostic and widespread use.

Conclusion

Abortion of dairy cattle presents serious challenges to the livestock industry, affecting both economics and animal welfare. This complex problem necessitates novel diagnostic approaches. Although traditional approaches have advantages, they frequently fail to properly capture complex microbial interactions at work. Recent advances in molecular and metagenomic approaches have significantly improved the detection and identification of abortion-causing agents. Metagenomic techniques provide crucial information about the microbiome associated with abortion. Using these cutting-edge methods, researchers and veterinarians can develop more effective approaches to diagnosing, preventing, and managing abortion in dairy cattle, thereby boosting herd health and productivity.

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Conflict of interest

The authors declare no conflicts of interest.

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Authors' contributions

NH, SS, EH, and AS developed the concepts and drafted the manuscript and the final approval.

Data availability

All data required to substantiate the findings of this research are included in the manuscript.

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