Review Article

Research progress on the structure and function of the main glycoproteins of bovine infectious rhinotracheitis virus

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Abstract

Infectious bovine rhinotracheitis (IBR) is an acute, febrile, and contact infectious disease. *Bovine infectious rhinotracheitis* is endemic in China and even the world, which not only restricts the development of the cattle industry but also brings significant economic losses to farms. *Infectious bovine rhinotracheitis virus (IBRV)* contains 25 - 33 structural proteins, of which glycoproteins gB, gC, gD, gE, gG, gH, gI, gK, gL, and gM are the major structural glycoproteins of *bovine rhinotracheitis virus*, and all of these glycoproteins are interconnected with the assembly, invasion, release, and replication of the virus, and the only glycoproteins that are known to have been identified are gB, gC, gD, and gE. Therefore, this paper focuses on an overview of the four glycoproteins, gB, gC, gD, and gE, of *bovine infectious rhinotracheitis virus (IBRV)*, with a view to providing a theoretical basis and reference for the differential diagnosis of IBR and the in-depth study of *IBRV*.

Keywords: *Bovine infectious rhinotracheitis*, *Bovine rhinotracheitis virus*, functionality, research progress, structural protein

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Introduction

Bovine infectious rhinotracheitis, also known as "necrotizing rhinitis" and "red nose disease", is a category 2 disease that must be inspected on entry into the country and is caused by the *Bovine infectious rhinotracheitis virus*, also known as *Bovine herpesvirus type I (BHV-1)* (Wang, 2020). *IBRV* is a double-stranded DNA virus with a capsule, belonging to the subfamily of herpesviruses (Herpesviridae), and consists of a core, capsid, and capsule, with the core consisting of a double-stranded DNA and proteins intertwined with each other, **Figure 1**. The main infectious host of *bovine infectious rhinotracheitis virus (IBRV)* is cattle. *IBRV* can infect cattle of different breeds and ages, while calves have higher infection and mortality rates and obvious neurological symptoms. Clinically, the disease can be divided into five types, namely respiratory tract type, reproductive tract type, conjunctivitis type, encephalitis type, and abortion type, which are mainly characterized by invasion of the respiratory tract, accompanied by rhinitis and dyspnea. Invasion of the reproductive system with clinical symptoms such as glans, mastitis, pustular vulvovaginitis, and abortion (Wang *et al., 2006*). Invisible infection of cattle is the most important source of infection of the disease, which can be transmitted through droplets, vectors, artificial insemination, and blood-sucking insects, such as inter-contact transmission, but also through mating, the affected cattle and other direct transmission (Dai *et al*., 2023).

The overall health of the herd is directly proportional to the feeding management and environmental conditions of the herd, and the mortality rate of cattle infected with the disease in good health is low, and most of them are transiently infected without obvious symptoms, which need to be diagnosed by serological tests (Liu *et al*., 2023). The characterization of *IBRV* infection of host cells is inextricably linked to its induction of pathological responses in host cells and to the viral proteins it contains (Paaperi *et al*., 2014). Once a cow is infected with *IBRV*, it can remain latent in the nervous system such as the trigeminal nerve for a long period of time, even if the cow recovers from the disease, sacral ganglia (Ostler *et al*., 2023). Ackermann's research has shown that *IBRV* has been shown in the trigeminal ganglia of intranasally infected calves during the latent stage of the infection, and *IBRV* DNA was detected in 13 of 23 trigeminal ganglia of latently infected calves. Single neurons harboring IBRV were observed in 4.9% of the sections (n = 325) of the trigeminal ganglia, which corresponds to the results in infected mice. The reproductive system infections should be demonstrated by calves with latent infections in the sacral ganglia (Ackermann *et al*., 1982). Narita reported histological alterations in sacral ganglia after experimental vaginal inoculation with IBRV, exhibits focal gliosis, and perivascular cuffing were present in the sacral spinal cords and sacro-lumbar spinal ganglia. This suggests that lesions in the sacro-lumbar spinal cord and ganglia indicated a close association with viral replication in the vaginal mucosa. Microscopical lesions in the sacro-lumbar spinal cords and ganglia and trigeminal ganglion are important in diagnosing IBR virus infection (Narita *et al*., 1978).

Under the stimulation of immunosuppression, transportation and feeding management irregularities, etc. can activate the virus latent in the sick cattle so that it re-exports to the outside world, etc., and then cause the outbreak of respiratory and reproductive tract infections (Winkler *et al*., 2000). The disease is difficult to eradicate due to the long-term or even lifelong carrying of the virus by diseased cattle, which to a certain extent limits the development of the cattle industry and brings about certain economic losses (Xu *et al*., 2010).

IBRV contains 25-33 structural proteins, of which gB is the most conserved among herpes viruses and is required for viral replication (Cao *et al*., 2015). gC is important for virus adsorption to tissue culture cells, and the expression of gC in cells does not have an effect on the number and presence of viral etiolated spots. gD is associated with virus penetration and entry into host cells (Wei ,2019). gE is the major glycoprotein on the viral vesicle membrane and is important in the replication of infected cells and the release of viral particles (Wu, 2020). Immunological tests have shown that cellular immunity induced by gD is not only stronger but also more durable than that induced by gB and gC. *IBRV* was first discovered in the United States in the 1950s and has continued to spread widely around the world, but only a few countries in Europe have eliminated the disease by culling and banning vaccinations, which has also caused huge economic losses (Cheng, 2021). However, these methods are not applicable to China, such a huge number of cattle breeding countries, the disease was introduced to China in the 1970s, greatly jeopardizing the development of the cattle industry and serological investigations. Many areas in China have varying degrees of infection, and the infection rate is rising year by year trend (Wang, 2019; Yang *et al*., 2012).

Therefore, an in-depth understanding of the relationship between the structure and function of *IBRV* proteins can help develop new vaccines and provide new ideas for preventing and controlling the disease.

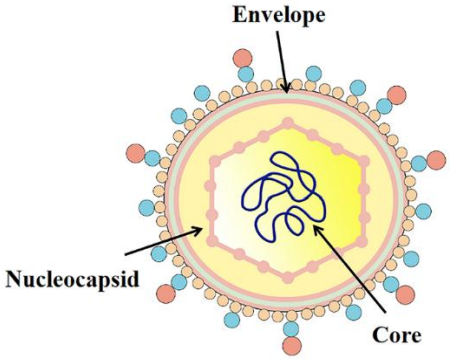

Figure 1 BHV-1 Virus structure diagram

Genomic Structural Features

Bovine herpesvirus 1 (BHV-1) is a member of the α-herpesvirus subfamily and can be classified into three subtypes by antigenic and genomic analyses, namely *BHV-1.1*, *BHV-1.2a* and *BHV-1.2b* (Zhang, 2012). *BHV-1.1* subtype strain is the pathogen of *bovine infectious rhinotracheitis (IBR)*. The clinical manifestations of *BHV-1.2a* and *BHV-1.2b* subtypes are often associated with *Infectious rhinotracheitis (IBR)*, Infectious pustular external vaginitis, and infectious pustular external vaginitis. *Infectious pustular external vaginitis (IPV)* and *Infectious pustular balanitis (IPB)* are associated with clinical abortion symptoms (Liu *et al.*, 2020). The *BHV-1* genome is a double-stranded DNA with a total length of 135.3 kb and a G+C content of up to 72%. It consists of a long unique region (UL, approximately 106 Kb) and a short unique region (US, approximately 10 Kb), as well as two inverted repeat sequences (IRS and TRS, each 11 kb), **Figure. 2**. IRS and TRS are located on both sides of the short independent zone, with the same sequence but opposite directions, so the short zone can reverse direction. The coding region of the genome accounts for approximately 84%, encoding approximately 70 proteins. During DNA replication, UL and US regions can reverse the direction of their unique regions, resulting in four isomers of linked DNA structures (Alves *et al.*, 2014). The US coding region in *BHV-1* can be divided into four glycoproteins: gE (US8), gI (US7), gG (US4), and gD (US6). The UL coding region can be divided into six glycoproteins: gB (UL27), gC (UL44), gH (UL22), gK (UL53), gM (UL10), and gL (UL1).

Leung-Tack P *et al*. completed the genome sequencing of *BHV-1* in 1955, with its coding region accounting for 84%. *BHV-1* has 72 open reading frames (ORFs). After comparing the genes of *Herpes simplex virus-1 (HSV-1)* and *BHV-1*, it was found that the UL0-5 gene in *BHV-1* is very unique, and its uniqueness lies in the fact that UL0-5 is different from all other genes α Members of the herpesvirus subfamily all have genes from different sources, and even *bovine herpesvirus 5 (BHV-5)* with an amino acid homology of up to 82 % with *BHV-1* has not been found to have a homologous gene with UL0-5 (Leung-Tack *et al*,.1994). The genes for *BHV-1* are categorized into essential and non-essential genes, but these two classifications are not absolute, as Schroder et al. discovered, under specific conditions, other viral proteins can replace the function of gD genes in the cross-cellular entry and transmission of viruses (Schroeder *et al.*, 1954).

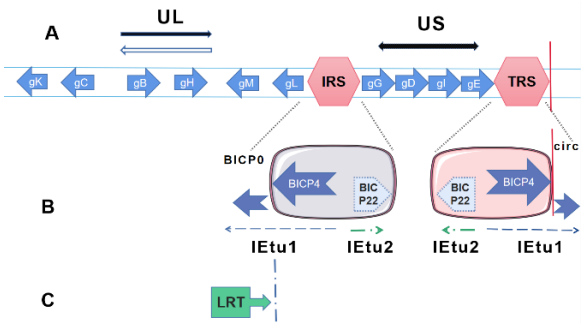

Figure 2 BHV-1 genome structure diagram

Structural Proteins

gB (UL27): The gB protein is composed of 932 amino acids and has a size of 130 ku. It can produce two subunits, gBb (75 kDa) and gBc (55 kDa), located in the long independent region (UL) of *IBRV*. It is a viral envelope component, N-glycosylated, and has high immunogenicity. gB protein is an essential protein with high conservation, participating in virus adsorption, invasion, cell fusion, and diffusion. The two small fragments cleaved by the enzyme exist in the form of heterodimers and interact with heparan sulfate-like receptors. The gene encoding gB protein has a total length of 2850 bp and is located in the UL27 region of the *BHV-1* gene. It is the most conserved gene among herpes virus coding genes and plays an indispensable role in its function. It can facilitate the connection between the viral envelope and the cell membrane, as well as allow infected cells to infiltrate the uninfected cell membrane (Li, 2016). Miethke A *et al*. Zhang Min showed through experiments that *IBRV* gB protein can replace the function of porcine *Pseudorabies virus (PRV)* gB protein, and the two have a one-way complementary pattern in function. By enhancing the neurotoxic effect of *PRV*, the pathway of toxin invasion into the nerves can be altered (Miethke *et al.*, 1995). Gerdts V *et al*. showed through experiments that the fusion protein formed by the c terminus of *IBRV* gB protein and the n terminus of *PRV* gB protein could form plaques in *BHV-1* and *PRV* gB defective strains and could not form plaques otherwise. This suggests that differences in the C-terminal of the protein lead to functional differences between the two proteins, and the C-terminal of the protein plays a decisive role in virus transmission (Gerdts *et al.*, 2000). The binding of the gB protein to cells is equivalent to a ligand- and receptor-specific reaction and is essential for viral replication (Babiuk *et al.*, 1996)

The gB protein of *Herpes Simplex Virus (HSV)* is very similar to that of *IBRV*, but the former did not undergo protease treatment after oligomerization. Most other *a-herpesvirus* gB proteins undergo cleavage by cytosolic Flynn protease at the RXK/RR site of the breakpoint motif, followed by disapplied linkage, resulting in the formation of a heterodimer consisting of 2 subunit links (Kopp *et al.*, 1994). However, the function of the *IBRV* viral gB protein, which has been hydrolyzed by Flynn protease, is not necessarily affected (Bender *et al.*, 2007). Insertion of the gene for *HSV-1* into the *IBRV* gB gene revealed that the recombinant *HSV-1* strain did not change its biological characteristics, whereas the *HSV-1* gB-specific monoclonal antibody had a reduced ability to neutralize recombinant *HSV-1* (Li *et al.*, 2006). Due to the high immunogenicity of the gB protein, the evolutionary relationship between herpes viruses can be determined by sequence comparison, and regional primers can be selectively designed as a method to detect and diagnose *IBRV*. Guo Liangshuai *et al*. constructed the prokaryotic recombinant expression plasmid pET-32a-gB by sequencing the major antigenic region of the gB gene and induced the expression of the plasmid after transforming it into BL21(DE3) receptor cells, and verified that the recombinant gB protein was

mainly expressed in the form of inclusion bodies. The successful in vitro prokaryotic expression and characterization of the IBRV gB gene lays the foundation for further studies on the preparation of suitable monoclonal antibodies (Guo *et al*., 2023). He Xiaoli *et al*. using the constructed gB recombinant strain, *Isopropyl β-D-Thiogalactoside (IPTG)* induced expression was carried out, and conditions such as action time and concentration of *IPTG* induced expression were optimized. The results showed that the purified recombinant gB protein could be recognized by standard *IBR*-positive sera, suggesting that the expressed target proteins are well reactive and can be used as specific antigens for *IBRV* detection. This test provides theoretical support for the clinical diagnosis of bovine infectious rhinotracheitis and the study of the specific function of the gB protein (He *et al*., 2019).

The construction of *IBRV* gB recombinant protein confirmed that the protein can be used for the preparation of *IBRV* monoclonal and multiclonal antibodies. gB recombinant protein construction also contributes to the establishment of the gB-ELISA diagnostic method and so on. The above experiments provide a better aid for further study of the structure and function of gB protein and provide a certain reference for the clinical diagnosis, prevention, and control of *IBR*.

gC (UL44): The gC protein is composed of 508 amino acids, the size of the expressed protein is 91 Ku, it is a viral vesicle component, N-, O-linked glycosylated, and highly immunogenic. The gC protein is non-essential, and haemagglutinin is closely related to viral virulence. Its binding acetyl heparin sulfate-like receptor binds to C3b complement factor, which interacts with it. The gC protein directs the initiation of viral adsorption by recognizing glycoproteins associated with the cell surface, facilitates the second process of *IBRV* invasion of the target cell, and also plays an important role in the replication of the virus (Quintero *et al*., 2023).

Ravishankar C *et al*. collected unilateral corneas and large amounts of plasma tear secretions from cows with conjunctivitis on a farm in India for virus isolation and then inoculated the supernatant obtained with *MDBK* cells and tested for *IBRV* antibody in serum containing 2 % fetal bovine serum using an indirect ELISA (I-ELISA) kit, which was positive. The gC gene was amplified by PCR and the gene was sequenced to determine the subtype of the *IBRV* isolate, and the nucleotide sequences obtained were analyzed by BLAST to confirm the presence of the *IBRV*-specific gene. Studies have shown that the gC protein induces a cellular immune response (Ravishankar *et al*., 2012). Zhang Fan *et al*. synthesized the gC gene by referring to the *IBRV* gene sequence registered in GenBank and successfully constructed the eukaryotic expression plasmid pCDNA4-gC-His. The plasmid was transfected with *MDBK* cells for expression, and the expressed protein was identical to the reactivity of the natural *IBRV* gC protein. This approach involves adding signal peptide sequences to gC proteins and guiding them for extracellular secretion expression, verifying the reactivity of eukaryotic recombinant gC proteins. This not only provides reference value for clinical target diagnosis but also guarantees the establishment of subsequent immunological diagnostic methods (Zhang *et al*., 2018a). Chen Ru *et al*. amplified the gC gene of the Bartha Nu/67 strain of *bovine infectious rhinotracheitis virus*. Insert gCd into the prokaryotic expression vector pET32a and construct a recombinant expression plasmid pET32a-bhv1gCd. Protein electrophoresis of the culture of recombinant plasmid transformed *E. coli* BL21 (DE3) detected the target product with a protein molecular weight of about 45 ku. The results of the immunoblotting test showed that the gCd recombinant protein had a specific reaction with the bovine infectious rhinotracheitis standard positive serum, indicating that the gC antigen active region fragment was expressed in prokaryotic cells and had good antigenicity. The purified recombinant protein can not only be directly applied to establish ELISA diagnostic methods but can also be used to prepare polyclonal and monoclonal antibodies against the gC gene or as antibodies for antigen screening and identification, further establishing immunological methods such as ELISA-based on monoclonal antibodies or multi antibodies to detect *IBR* pathogens (Chen *et al*., 2008).

The gC protein is relatively highly expressed in *IBRV* and induces both humoral and cellular immunity. This provides a reference for clinical target diagnosis and provides some research direction for establishing immunological diagnostic methods. Target diagnosis is more accurate and rapid than traditional diagnosis, which provides strong support for the clinical diagnosis of *IBR*.

gD (US6): The gD protein is composed of 417 amino acids, the size of the expressed protein is 71 Ku, it is a viral vesicle component, N-, O-linked glycosylated and highly immunogenic. The gD proteins are conditionally essential proteins, the predominant structural and immune proteins of *IBRV*, and function to diffuse between cells and may interact with gH. The gD protein recognizes 6-residual mannose phosphate and binds to its receptor, unlike the acetyl heparin sulfate-like receptor. The gD protein is essential for *IBRV* to infect cells and replicate itself, allowing the body to develop strong cellular and humoral immunity, and the protein plays an important role in both the pathogenesis of *IBRV* and the induction of antibody production (Zheng *et al*., 2000; Hanon *et al*., 1999).

Levings R L *et al*. performed virus-neutralizing and indirect fluorescent antibody assays for the reactivity of three *bovine monoclonal antibodies (BomAb)* against two *bovine herpesvirus 1.1 (IBRV-1.1)* isolates. The results showed that anti-gD *BomAb* reacted strongly with all tested isolates of *IBRV -1.1* and *IBRV -1*. Therefore, gD proteins are clinically important for both the detection of *IBRV* infection and the monitoring of immune antibodies (Levings *et al*., 2015). Studies have shown that compared to other glycoproteins, antibodies produced by stimulating the gD protein can neutralize the virus as much as possible. On this basis, the gD protein is considered a candidate target for the development of *IBRV* diagnostic products or antiviral drugs (Khattar *et al*., 2010) Liu Bing established an

indirect ELISA method for the detection of *IBRV* antibodies developed using ELISA plates coated with prokaryotic ally expressed gD protein with good reactivity. The results of the clinical application showed that the method can be used to detect wild virus infection antibodies and immunological antibodies in *IBRV*. The above methods provide inexpensive, simple, rapid, and accurate serological assays for the clinical detection of *IBRV* in China (Liu *et al., 20*23). Ding Guowei *et al*. primers were designed according to the registered *IBRV* gD protein sequence in the NCBI database, and the immunoglobulin structural domain of the gD gene was amplified by PCR. pET32a -Δ gD vector, which was efficiently expressed in Rosetta (DE3), was constructed, transformed into *Escherichia coli* Rosetta (DE3) receptor cells, and recombinant protein expression was induced. By sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis, the target protein with a relative molecular mass consistent with the theoretical relative molecular mass of the fusion protein was obtained. The concentration of gD recombinant protein purified by nickel column was above 95%, and the protein concentration was 2.5 mg/mL. An indirect ELISA assay for the detection of anti-*IBRV* antibodies was established using the high-purity purpose gD protein as antigen and *Horseradish peroxidase (HRP)*-labeled rabbit anti-bovine IgG as the secondary antibody, and the results showed that the recombinant protein had good reactivity, and could be used for the promotion of the clinical application of the *IBRV* antibody detection kit (Ding *et al*., 2018).

Although the molecular biology test for *IBRV* has advantages in clinical diagnosis, it has the disadvantages of high cost and high operation level requirement, which makes it unsuitable for large-volume testing and screening. The above test establishes an indirect ELISA method for detecting *IBRV* antibodies by constructing gD recombinant proteins, which facilitates better service for clinical diagnosis. Compared with the gB-ELISA method established in the above test, the indirect ELISA uses an enzyme-labeled secondary antibody, which has higher sensitivity and requires only a small amount of labeled antibody, which is more cost-effective.

gE (US8): The gE protein is composed of 575 amino acids and the size of the expressed protein is 92 Ku. It is a viral vesicle component and N-linked glycosylated. The gE protein is non-essential and interacts with Gi (Fc receptor), which diffuses between cells and is the main virulence factor of the virus, and its virulence factor correlates with invasive neurological effects; deficiency of gE may result in decreased virulence but does not affect the immunogenicity of the virus. The gE protein is suitable as an antigenic marker because of its good immunogenicity.

Yang Mujiao *et al*. amplified the full-length gE gene by PCR to construct the recombinant plasmid pFB-gE. Transforming the correctly sequenced pFB-gE into DH10Bac receptor cells, obtaining a recombinant plasmid (rBacmid gE), and then immunizing four mice twice with the recombinant plasmid (rgE), and detecting antibodies in mouse serum through indirect ELISA. The results showed that the recombinant plasmid (rgE) induced the production of specific antibodies in mice, suggesting that rgE has good immunogenicity. This study lays the foundation for the study of *IBRV* gE protein structure and function (Yang *et al., 20*22). Zhang Fan *et al*. Fan Zhang prepared gE polyclonal antibody by purifying *IBRV* and recombinant gE protein and transfected *MDBK* cell line with nickel column to purify recombinant gE protein with His-tag after correctly identifying by bis-enzymatic digestion. The purified recombinant gE protein was subcutaneously injected into New Zealand white rabbits at multiple sites to prepare and purify polyclonal antibodies, and its antigenicity was analyzed using dot blot hybridization and indirect ELISA. This experiment lays the foundation for the development of *IBRV* vaccines (Zhang *et al., 20*20b). Stefano Petrini *et al*. inoculated commercial inactivated gE deletion marker vaccines into pregnant heifers to be evaluated for passive immunization of calves after parturition. Twelve pregnant cows lacking neutralizing antibodies to *IBRV* were inoculated in this experiment, and six of them were divided into groups. Experimental results showed that the inactivated gE deletion-labeled vaccine was safe and also produced a good humoral immune response, with passive immunity persisting up to PCD180 from pregnant cows to calving and 180 days post-calving (PCD180) and in calf serum. Stefano Petrini *et al*. will conduct further studies to evaluate whether passive immunization induced by these vaccines protects calves against strong strains of *IBRV* (wt) (Petrini *et al., 20*20). Liu Huanqi *et al*. designed primers specific for the gE gene amplified the gE gene by PCR and constructed the recombinant expression plasmid pET-32a-gE. Expression was induced by IPTG in the obtained positive colonies and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for gE protein expression and purification. The results showed that the recombinant protein was purified using Ni chromatography column, and concentration of the purified gE recombinant protein was 0.4 mg/Ml as identified by Western-blot and ELISA, and the pET-32a-gE fusion protein was successfully expressed in Escherichia coli (E. coli), and the purified recombinant protein had good reactivity as analyzed by immunoblotting (Liu *et al., 20*15).

gE as a non-essential gene for replication, favoring the retention of the complete spatial structure and biological activity of the expression product. The above tests verified that the gE gene has good immunogenicity, which lays a solid theoretical and practical foundation for the establishment of clinical diagnosis, the development of vaccines, and the related research on the pathogenic mechanism of *IBRV*.

Conclusion

Bovine respiratory disease syndrome (BRDC) is caused by pathogens such as *Bovine Respiratory Syncytial Virus* (*BRSV*), *Bovine Parainfluenza Virus 3 (BPIV3)*, and *Bovine Herpesvirus 1 (BHV-1)*, of which *BHV-1* is one of the major etiologic agents responsible for bovine mortality (Kirchhoff *et al*., 2014; Nandi *et al*., 2009). *Bovine infectious rhinotracheitis* is widely spread all over

the world, the disease is very harmful to the cattle industry, and the incidence of the disease is increasing year by year, causing great attention at home and abroad and in-depth research on it (Wang.,2006). Therefore, the control of IBR is an urgent problem that needs to be solved. Establishing rapid and high specificity and vaccine immunization is the best way to prevent this disease.

It has been reported that animals immunized with *IBRV* gB, gC, and gD proteins can produce high levels of antibodies, and the protective effect of a single glycoprotein on the experimental challenge is stronger than that of commercial inactivated vaccines (Babiuk *et al., 1987*). Hou Lina *et al*. made a water-in-oil subunit vaccine mixing gB with Freund's adjuvant was prepared to immunize guinea pigs and also combined the lesions in lung tissue, viral shedding, and the clinical symptom score to assess the vaccine validity. The results indicated that the gB can induce antibody in guinea pigs and safety (Hou *et al., 2022*). This demonstrates the potential of the gB protein as a vaccine candidate for *IBR*. It can help accelerate the elimination of *IBR*. Since the gC gene is a non-essential gene, the deletion of the gC gene can be used as a genetic marker in the construction of the gene deletion vaccine, TK-/gC-genetically engineered deletion seedlings, or even attenuated seedlings with a three-gene deletion, can be constructed to control the disease, and anti-gC monoclonal antibodies can also be used to identify deletion strains from wild strains (Beer *et al., 2003*). For DNA vaccines encoded by gC proteins, they can induce the production of neutralizing antibodies and the response of B cells. The *IBRV* attenuated vaccine, which can also be developed by missing the TK gene, has been put on the market. This vaccine does not undergo virulence regression, has high reliability, and has achieved good results (Gupta *et al., 2001*). The gB, gC, gD, and gE proteins of *IBRV*, as the main glycoproteins on the surface of the viral envelope, play important roles in virus adsorption and invasion of cells. As the main antigen protein of IBRV, gD protein can cause higher and more persistent cellular immunity than gB and gC proteins. Therefore, monoclonal antibodies prepared from gD proteins have a strong ability to neutralize viruses, and gD proteins can be used for the development of in vitro diagnostic reagents (Brownlie *et al., 2015*). Bian Yuchen *et al*. successfully established a preliminary colloidal gold immunochromatographic test strip for IBRV antibody detection using the double antigen sandwich method. The colloidal gold labeling and the protein-coated in the detection package are both gD proteins, which can distinguish the positivity of the test sample. The serum sample detected by this method does not need to be diluted or diluted, and the use of the same protein makes it more specific and less prone to false positives. It is more convenient, accurate, and economical to use in clinical practice, which makes it very suitable for the mass production of commercial test strips and provides a new detection method for rapid screening of clinical samples in grassroots IBR (Bian *et al., 2023*).

In European countries where *IBR* has been eradicated, gE gene deletion marker vaccines are widely used. gE gene deletion reduces the ability of the virus to spread from cell to cell, which leads to a reduction in the infectivity of *IBRV*, and the deletion of the gE gene can be used as a serological marker to differentiate between wild-virus infection and vaccine immunity (Letellier *et al., 2001*; Qiao.,2015). The gE gene, as a non-essential glycoprotein for virus replication both in vivo and in vitro, has a relatively small impact on virus replication and does not affect virus immunogenicity. The characteristics of the gE gene make it the primary choice for studying *IBR* vaccines (Zhu *et al., 2017*). Studies have shown that *IBRV* gE protein expressed and purified in prokaryotes can be used to prepare highly efficient and stable gE monoclonal antibodies, laying the foundation for the establishment of diagnostic methods and related basic research for *IBR* in the future (Zhao *et al., 2018*). At present, the vaccines developed on the market mainly include inactivated vaccines and gene deletion marker live vaccines. A safe and efficient vaccine can have a good preventive effect on the occurrence of this disease, as well as prevent a low fattening rate, low reproduction rate, and low milk production in cattle, greatly reducing the possibility of infected animals excreting toxins. Vaccination provides a guarantee for effective prevention and control of the disease (Khatoon *et al., 2018*; Xu *et al., 2012*).

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