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Development and Application of a Rapid Test for Detection of Bovine Viral Diarrhea Virus-specific Antibodies

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ABSTRACT

Bovine viral diarrhea virus (BVDV) is an economically consequential animal pathogen that engenders substantial financial losses in the realm of cattle farming, and it maintains a prevalent presence on a global scale. Presently, the prevailing modality employed for its detection is enzyme-linked immunosorbent assay (ELISA), which necessitates the use of sophisticated instrumentation, protracts the duration of analysis, and is primarily suited for laboratory-based diagnostics. Regrettably, ELISAs do not lend themselves to convenient on-site detection within cattle farms. To surmount this predicament, colloidal gold particles were synthesized using tri-sodium citrate reduction, and subsequently harnessed to label the E2 protein (E2-Au). By amalgamating the gold standard binding release pad with colloidal E2-Au, we have devised a comprehensive assay system. The nitrocellulose membrane serves as the platform, whereby the detection line is adorned with E2-Au, while the quality control line is coated with proprietary polyclonal rabbit antibodies specific to E2. The method yields results within a span of 10-15 minutes, exhibiting an impressive total compliance rate of 93.36% (239/256) when compared to the commercial kit. The E2 test strip demonstrates specific reactivity with the anti-BVDV antibody, while avoiding any cross-reactivity with antibodies targeting Brucella, bovine foot and mouth disease, Mycobacterium bovis, bovine para-tuberculosis, bovine pasteurellosis, and bovine infectious rhinotracheitis. Consequently, the E2 strips offer heightened specificity, cost-effectiveness, and ease of operation, rendering them more convenient and expedient in comparison to ELISA kits. Utilizing the E2 strips, a comprehensive assessment encompassing 36 cattle farms and 2035 cattle in the vicinity of Xinjiang, China, was conducted. The positive rate within the group reached an impressive 91.67%, with individual positive rates standing at 54.05%.

Key words: Bovine viral diarrhea virus; Colloidal gold; Test strip; Epidemiological investigation, Bovine disease

INTRODUCTION

Bovine viral diarrhea (BVD) stands as the most significant and widespread infectious endemic disease affecting the global cattle population, causing substantial economic losses (Cowley et al. 2014). BVD exhibits a high prevalence, persistent nature, and consequential clinical impact, making it a paramount infectious disease within the livestock industry (Su et al. 2023). BVDV, a member of the Pestivirus genus within the Flaviviridae family, is characterized as a single-stranded, positive-sense RNA virus, alongside border disease virus (BDV) and classical swine fever virus (CSFV) (Ridpath 2003). Based on the Flaviviridae Study Group's recommendations from the International Committee on Taxonomy of Viruses (ICTV), BVDV can be categorized into three genotypes: BVDV-1 (Pestivirus A), BVDV-2 (Pestivirus B) and an atypical Pestivirus also known as BVDV-3 (Smith et al. 2017). The genome of BVDV spans approximately 12.3 kilobases (kb) and consists of a single-stranded, positive-sense RNA molecule. It encompasses a single open reading frame (ORF) that encodes around 4000 amino acids, flanked by untranslated regions (UTRs) at both the 5' and 3' ends (Meyers and Thiel 1996). The viral polyprotein (NH2-Npro-C-Erns-E1-E2-p7-NS2-3-NS4-NS4B-NS5A-NS5B-COOH) is processed through the activity of viral and cellular proteases, resulting in the generation of structural and non-structural proteins (Dubrau et al. 2017).

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Among the structural proteins, the capsid protein C and three envelope glycoproteins, namely Erns, E1 and E2, are present. E2, the predominant envelope glycoprotein within BVDV, holds significant immunogenicity and serves a pivotal role in receptor attachment for host cell entry (Mv 1995). Following natural infection, the immune response primarily targets the viral protein E2, while generating a comparatively weaker response to the viral proteins Erns and E1 (El Omari et al. 2013). Nevertheless, studies have indicated that the average annual production loss attributable to BVDV infection ranges from €42.14 to €67.19 per animal (Pinior et al. 2019). In Australia, the economic impact of BVDV can escalate to AUD 50.9 million annually (McGowan et al. 2020). Currently, various detection methods are employed for the diagnosis of BVDV, including virus isolation, ELISA, real-time PCR, and serological tests (Hansen et al. 2015).

Traditionally, the virus neutralization test (VNT) has served as a reference test for serological diagnosis of BVD. However, this method is time-consuming, laborious, resource-intensive, requires cell culture facilities, and entails the handling of live virus, posing certain risks (Kalaiyarasu et al. 2015). ELISA (Robiolo and Schauer 2007) and real-time PCR (Larska and Polak 2011) also necessitate specialized instruments, prolonged analysis time and are primarily suited for laboratory-based diagnostics. Unfortunately, these methods are not conducive to on-site detection in cattle farms.

To address these limitations, a novel in vitro diagnostic technique called Gold immune-chromatography assay (GICA) has emerged. Developed in the 1990s, GICA combines colloidal gold labelling technology, immuno-chromatography, and new material technology (Shyu et al. 2002). This technique utilizes visually detectable labels, such as colloidal gold or dyed latex, which exhibit intuitive experimental phenomena, such as color development. GICA offers several advantages, including rapid and straightforward operation, absence of specialized instrumentation, accurate and sensitive results, and the ability to be transported and stored at room temperature. Consequently, GICA has found extensive applications in various fields, including biomedicine and epidemiological investigations.

In this experimental setup, gold nanoparticle-labelled E2 protein (E2-Au) was synthesized through the meticulous reduction of gold ions by tri-sodium citrate. This method ensures the formation of stable gold nanoparticles conjugated with the BVDV E2 protein. Subsequently, the E2-Au complexes, in conjunction with a proprietary polyclonal rabbit anti-E2 antibody, were precisely sprayed onto a nitrocellulose membrane. The nitrocellulose membrane serves a dual role as both the detection line, where E2-Au complexes are immobilized, and the quality control line, where the proprietary polyclonal rabbit antibody is affixed. The methodological precision in the application and drying of these components on the membrane is crucial for ensuring the uniformity and reliability of the diagnostic assay.

This innovative approach culminates in the development of a rapid anti-BVDV antibody detection method that is not only exceptionally convenient and expeditious but also ideally suited for on-farm applications. The utilization of gold-labelled E2 protein enhances the sensitivity of the assay, providing a robust means of detecting BVDV antibodies. The nitrocellulose membrane, with its dual functionality, facilitates the efficient capture of antibody-antigen complexes, further contributing to the reliability of the diagnostic method.

Beyond its diagnostic utility, this method also offers an additional advantage by facilitating BVDV purification within herds. The specific targeting of the E2 protein and the gold-labelled detection system enable precise and rapid identification of BVDV infections. This dual functionality not only aids in diagnostic efforts but also supports targeted purification strategies within cattle herds.

MATERIALS AND METHODS

Ethical approval

It is important to ensure that research on bovine viral diarrhea is conducted with the utmost respect for the well-being of the animals involved, adhering to international ethical guidelines and minimizing any potential harm.

Construction of the expression plasmids

The amino acid sequence of the E2 pro-protein was meticulously optimized utilizing the advanced codon optimization software, MaxCodonTM Optimization Program (V13). Subsequently, the E2 progene was seamlessly integrated into the expression vector pET30a, employing the precise cleavage sites NdeI and HindIII provided by restriction enzymes. The size of the released fragment was found to be in perfect alignment with the anticipated dimensions of the cloned insert. Furthermore, the accuracy of the procedure was validated through meticulous sequencing analysis, confirming the successful integration of the E2 gene into the pET-30a(+) vector, with the E2 gene maintaining its intended frame and devoid of any sequence errors.

E2 coding cloning, expression and reactogenicity identification

A flourishing colony of BL21 (DE3) cells harboring pET-30a(+) was meticulously gathered and introduced into a 4 ml culture medium of LB, fortified with 50μg/mL kanamycin (Kan-LB), for overnight propagation at a temperature of 37°C. The cultures, inoculated at a ratio of 1:100, were employed to initiate the growth of fresh Kan-LB cultures, which were incubated at 37°C for approximately 4 to 6hours until the optical density value of the cultures at OD600 nm reached approximately 0.5 to 0.8. Subsequently, IPTG was introduced to attain a final concentration of 0.1mM, followed by a 6-hour duration of induction. The expression of the recombinant protein was assessed via SDS-PAGE, with bovine serum albumin (BSA) serving as the standard. The expressed recombinant protein was meticulously purified utilizing affinity chromatography (Ni-IDA resin) and the resulting product was duly preserved (Jia et al. 2009). The immune-reactivity of the fusion protein was determined through Western blotting and ELISA techniques.

Preparation of colloidal gold nanoparticles

Colloidal gold nanoparticles were synthesized via the reduction of a solution containing chloroauric acid using tri-sodium citrate. Initially, a precisely measured volume of 100mL of fresh ultrapure water was used to prepare a

chloroauric acid solution with a concentration of 0.01%. After reaching boiling point, 1% tri-sodium citrate was swiftly added to a 1.2mL portion of the solution while vigorously stirring. The color of the solution underwent a progressive transformation from a faint yellow hue to a rich burgundy shade, eventually darkening to a deep black color, and finally settling into an exquisite wine-red hue. Once the color of the colloidal gold solution stabilized, it was continuously heated and boiled for 10min. Subsequently, the resulting gold nanoparticle solution was cooled to room temperature and stored at 40°C until further use. The morphology of the colloidal gold nanoparticles was examined using transmission electron microscopy, allowing for detailed visualization and characterization of their structure. Additionally, the size of the colloidal gold nanoparticles was calculated precisely. To determine the maximum absorption peak, the colloidal gold solution was subjected to scanning using an ultraviolet spectrophotometer within the visible light range of 400-700nm.

Conjugation of E2 protein to colloidal gold particles

The conjugation of the E2 protein to colloidal gold particles was conducted following previously established procedures. In summary, colloidal gold particles measuring 40-50 nm in size were employed for the conjugation process. A volume of 0.9mL of colloidal gold solution (1% w/v, pH 8.5) was gently combined with 0.1mL of E2 protein solution (1mg/mL) and subjected to shaking for a duration of 4-6 hours at room temperature. Following this, the colloidal gold particles were separated through centrifugation at 4°C for 30minutes, using a centrifuge with an 8178 swing-out rotor operating at 10,000rpm/min, specifically the Labofuge 400R model from Heraus Instrument, USA. The resulting pellet was then re-suspended in 1 ml of working buffer, which consisted of a 20mM Tris/HCl buffer (pH 8.2) containing 1% w/v bovine serum albumin (BSA). The prepared E2 protein colloidal gold probes were stored at 4°C until needed, with a recommended application volume of 30μL per square centimeter on the conjugation pad.

Preparation of immune-chromatographic test strips

The colloidal E2-Au, representing the gold standard binding release pad, was combined with the test line coated with colloidal E2-Au, while the control line was coated with a polyclonal rabbit antibody against E2. Each line had dimensions of 4 mm in length and 1 mm in width. The appropriate amount of colloidal E2-Au used was between 2 to 3μg, and the suitable coating amount of polyclonal rabbit antibody against E2 was also 2 to 3μg, applied onto high-quality nitrocellulose and dried at 37°C for 30minutes. Once dried, the coated proteins were affixed to a plastic polyethylene plate in a specific sequence, starting from the bottom to the top: the gold standard combined with the release pad, the nitrocellulose membrane, and the uppermost layer, followed by the absorbent pad. The assembly was then covered with tape and cut into strips measuring 4mm in width. Finally, all the components of the strip, including the red blood filter pad, sample pad, conjugate pad, nitrocellulose membrane, and absorbent pad, were meticulously layered onto a sheet of plastic backing (Fig. 1). A desiccant was included, and the strip was sealed at room temperature.

Sensitivity and specificity

The identical batch of test paper was employed to discern positive control bovine serum, negative control bovine serum, and samples containing antibodies against Brucella (BR), bovine foot and mouth disease (FMD), Mycobacterium bovis (MB), bovine paratuberculosis (Map), bovine pasteurellosis (BP), and bovine infectious rhinotracheitis (IBR). These aforementioned sera were previously ascertained via ELISA to exhibit positive antibody presence while testing negative for BVDV antibodies. In a concise summary, twofold serial dilutions of BVDV-positive control serum samples, acquired from the BVDV ELISA commercial kit, were singularly administered to the strips. The utilized solutions encompassed an empty white contrast solution, specifically a 0.01mol/L phosphate-buffered saline (PBS) with a pH of 7.4, as well as a gradient concentration sample solution featuring diverse dilutions, namely 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, and 1:128, derived from the BVDV antibody positive serum.

Comparison with commercial ELISA kits

To assess the performance of the E2 test strips, a total of 256 cattle serum samples obtained from various farms were subjected to testing using both the E2 test strips and a commercially available ELISA kit. The compliance rate of the E2 test strips was calculated based on the comparison with the results obtained from the commercial ELISA.

Stability test of test strips

For the stability evaluation of the test strips, three separate batches of E2 protein were purified and utilized in the preparation of the test strips. Each batch consisted of 20 individual test strips, which were stored under controlled conditions in refrigerators at 4°C and thermostats at 37°C. After a period of 2 weeks and 4 weeks, the BVDV-positive control serum and negative control serum (obtained from the BVDV ELISA commercial kit) were measured using the stored test strips.

Epidemiological investigation of BVDV infection

Simultaneously, an epidemiological investigation was conducted to assess BVDV infection. A total of 1935 bovine serum samples were collected from 36 herds located in the Shihezi, Shawan, and Kuitun areas of Xinjiang. These herds had not received BVDV vaccination. The collected samples were tested for the presence of BVDV antibodies, and the positive rate was calculated to determine the extent of BVDV infection in the investigated population.

RESULTS

Construction of the expression plasmids

The recombinant expression plasmid pET-30aE2 was subsequently generated through the ligation of the E2 gene (1,055bp) into a pET-30a vector (5,139bp). The fidelity of the ligation process was verified by employing the restriction enzymes NdeI and HindIII for digestion. The resulting fragment size corresponded precisely to the expected dimensions of the inserted DNA (Fig. 2a). Sequencing analysis further corroborated the successful integration of the E2 gene into the pET-30a vector, ensuring the preservation of its reading frame without any discernible sequence errors (data not shown).

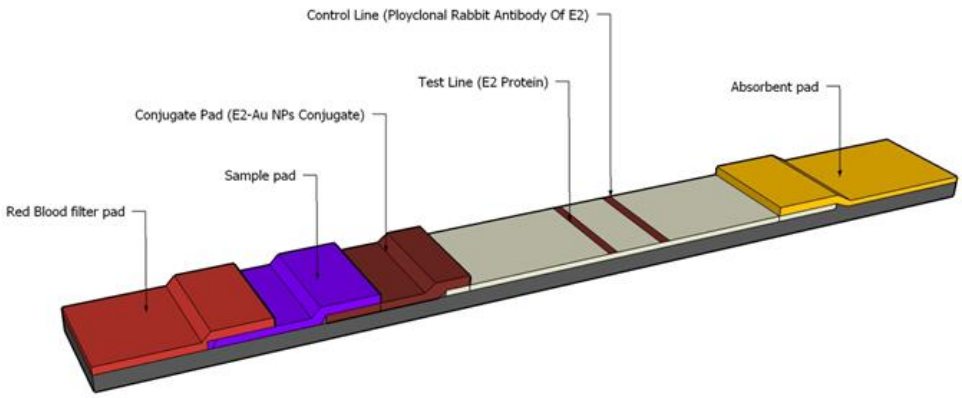

Fig. 1: Presents a schematic diagram illustrating the different components of the E2 strip.

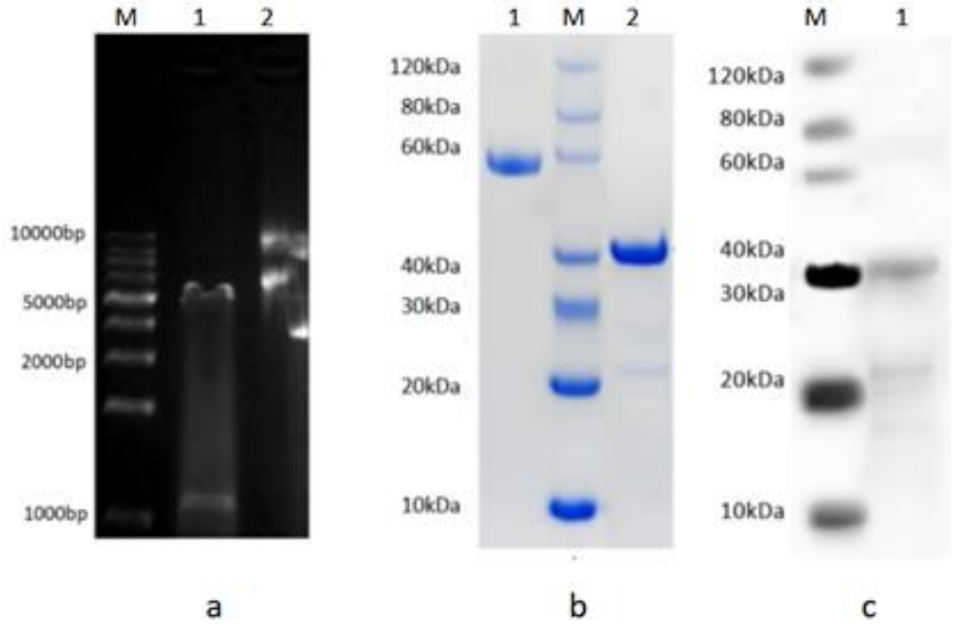

Fig. 2a: Lane M depicts the DNA marker, while Lane 1 exhibits the plasmid DNA. In Lane 2, the plasmid DNA was subjected to digestion with NdeI/HindIII, **b)** lane M represents the protein marker. Lane 1 showcases the protein derived from Bovine Serum Albumin (BSA). Lane 2 displays the purified E2 protein, and **c)** lane M signifies the protein marker, while Lane 1 specifically exhibits the E2 protein.

Expression and identification of the BVDV core protein

Induction of E2 expression was achieved by subjecting transformed E. coli cells to 1mM IPTG treatment at 37°C for 4hours. The recombinant E2 protein predominantly localized within the insoluble fraction, adopting the form of inclusion bodies. Subsequently, the insoluble fractions were solubilized and loaded onto a Ni2-agarose column. The immobilized protein was subsequently eluted and subjected to SDS-PAGE analysis. A discrete band, consistent with the anticipated molecular weight of E2 (39.3kDa), was readily discernible (Fig. 2b). Notably, the expressed product was meticulously preserved, and the concentration of E2 protein was quantified as 0.449mg/mL. Western blot analysis showcased the specific recognition of the Erns recombinant protein, produced through prokaryotic expression, by BVDV-positive serum antibodies (Fig. 2c), indicative of its robust immunogenic properties.

Quality identification of colloidal gold

The colloidal gold, synthesized through the trisodium citrate reduction method, exhibited a rich wine-red color. The solution appeared clear and transparent, devoid of any suspended particles. Electron microscopy analysis revealed the uniform dispersion of colloidal gold particles, characterized by relatively regular shapes, with the majority appearing round in nature (Fig. 3a). Spectrophotometric scanning within the visible range further demonstrated that the colloidal gold solution exclusively displayed a distinct absorption peak at 535nm (Fig. 3b), which corresponds to the characteristic absorption peak of colloidal gold. Notably, the peak exhibited a narrow shape, indicative of uniform gold particle sizes and the absence of particle agglomeration. As depicted in the particle size distribution map (Fig. 3c), the gold particles ranged in size between 20nm and 40nm. In conclusion, the colloidal gold synthesized in this experiment exhibited exceptional quality.

Development of the E2 ELISA

To investigate the immunogenicity of the E2 protein, checkerboard titrations were employed to determine the optimal working dilutions of the antigen and serum samples. Known positive and negative sera were subjected to twofold serial dilutions, yielding an assessment of the antigen-antibody interaction. Notably, the maximum disparity in OD450 values between the positive and negative sera (P/N=4.803) was observed when the antigen and serum dilutions were set at 2μg/mL and 1:50, respectively. Furthermore, meticulous optimization led to the identification of the ideal dilution for the rabbit anti-bovine HRP antibodies, resulting in a dilution ratio of 1:10,000 (Table 1). These outcomes unequivocally establish the antigenic nature of the E2 protein and its potential suitability for the fabrication of test strips.

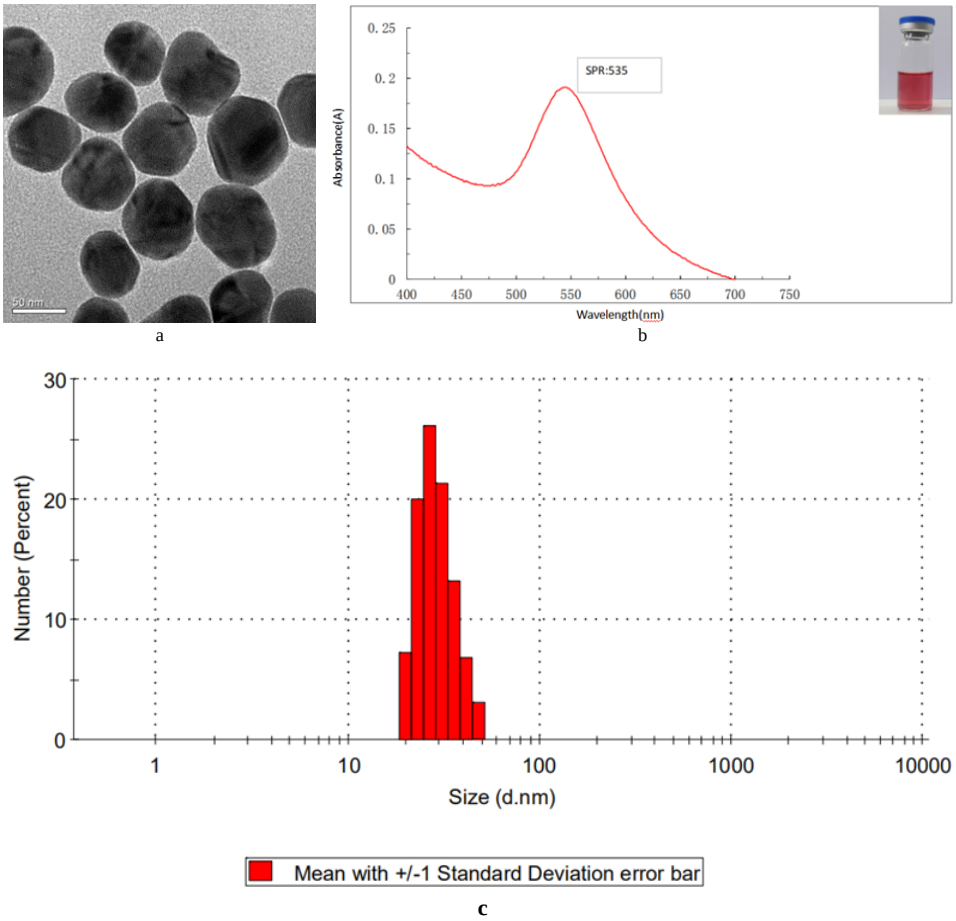

Fig. 3a: The transmission electron micrograph showcases the E2-Au particles, **b)** the spectrophotometer scan depicts the absorption spectrum of the E2-Au particles, and **c)** the particle size distribution map provides an illustration of the distribution of particle sizes in the E2-Au sample.

Table 1: Optimized Parameters for Indirect ELISA

	Coated antigen	Blocking	Serum	Anti-bovine IgG-HRP	Substrate Effect conditions
Optimized dilutions	0.2µg/well	5% Skim milk	1:100 Equine serum	1:10 000	
Reaction conditions	4 °C/16 h	37 °C/2 h	37°C/30 min	37°C/30 min	37°C/10 min

Sensitivity and specificity

In terms of sensitivity, the anti-BVDV antibody-positive serum was subjected to continuous twofold dilutions, starting from a 16-fold dilution. Notably, the test line retained its color even at this dilution level, indicating the persistence of detectable levels of the targeted antibodies. At a 32-fold dilution, however, the color development became weaker, and no color development occurred when both the blank control and serum were diluted beyond 32-fold (Fig. 4a).

To assess specificity, the E2 strips were exposed to various antibodies, including anti-BVDV, anti-BR, anti-FMD, anti-MB, anti-Map, anti-BP, and anti-IBR. The serum samples were diluted twice with PBS before applying them to the strips. After an incubation period of 10-15minutes, only the anti-BVDV serum yielded a positive result, exhibiting color development on the test line. In contrast, all other serum samples displayed negative results, with no detectable color development (Fig. 4b).

Comparison with test results of commercial kits

The robustness of our E2 test strip protocol was evaluated in a clinical diagnostic setting by comparing the results with an E2 ELISA. A total of 256 clinical serum samples were analyzed, and the levels of anti-BVDV antibodies were measured. The E2 test strips demonstrated concordant results with the E2 ELISA in 239 out of 256 samples,

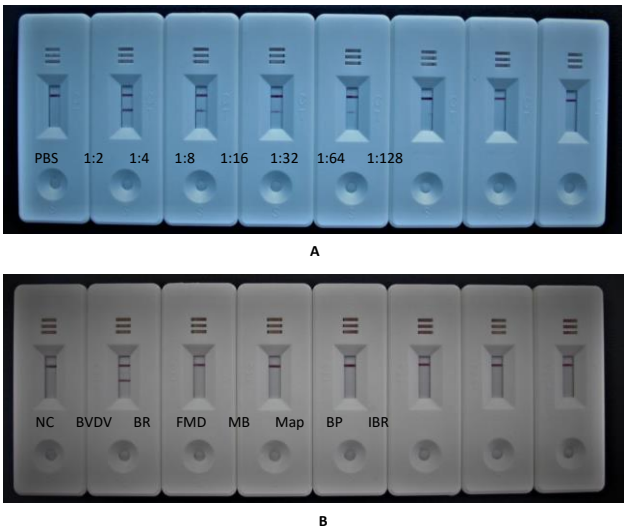

Fig. 4a: The sensitivity test involved continuous twofold dilution of anti-BVDV antibody-positive serum. Even at **A)** 16-fold dilution, the test line on the E2 strips remained colored, indicating the presence of detectable antibodies. However, when the serum was diluted beyond 16 times, the antibodies could not be detected, and **B)** to assess cross-reactivity, the E2 strips were exposed to various antibodies, including anti-BVDV, anti-BR, anti-FMD, anti-MB, anti-Map, anti-BP, and anti-IBR. Among these, only the anti-BVDV antibody resulted in the appearance of a red band in the test region, indicating specificity to the target antigen.

yielding a congruence rate of 93.36%. Specifically, out of the 158 samples that tested positive for anti-BVDV by the E2 ELISA, 146 samples were also positive by the E2 test strips. Similarly, 98 samples that tested negative by the E2 ELISA were confirmed as negative by the E2 test strips in 93 cases. These findings substantiate the effectiveness and reliability of our E2 test strips as a diagnostic tool for the detection of BVDV.

Repeatability and stability test of strips

To assess the repeatability and stability of the test strips, positive control serum and negative control serum were subjected to testing using three different batches of test strips, with each batch tested three times. The results of the repeated tests consistently yielded consistent outcomes, indicating excellent repeatability of the test strips.

Epidemiological investigation of BVDV infection

The results of a comprehensive epidemiological investigation conducted on 64 herds in Xinjiang Province are presented in Table 2. It was found that 89.62% of the herds tested positive for BVDV antibodies, with an individual positive rate of 61.88%. Among the five different areas examined, Shihezi, Manasi, Kuitun, and Ili exhibited varying levels of BVDV infection, with a 100% positive rate observed. Notably, Manasi had the highest group positive rate (100%) and individual positive rate (67.40%) among the five areas. In contrast, Shihezi had the lowest herd positive rate (100%) and individual positive rate (43.62%) among the five provinces (Table 3). Additionally, the investigation encompassed 18 large-scale cattle farms and 15 free-range cattle herds, with the respective results presented in Table 4. The large-scale cattle farms demonstrated a 100% positive rate, while retail farms exhibited a group positive rate of 83.33%. Interestingly, the individual infection rate was significantly higher in large-scale cattle farms (61.76%) compared to retail farms (17.97%). Furthermore, serum samples were collected from cattle with different purposes, and the anti-BVDV antibody test results are depicted in Table 5. Among the different categories, milk cow serum samples displayed the highest positive rate for anti-BVDV antibodies (66.05%), surpassing the rates observed in meat bovine (40.37%) and breeding bovine (38.69%) serum samples. The investigation also encompassed different age groups, namely calves (before 6 months old), young cattle (6-18 months old), and adult cattle (18 months old and older), with the results presented in Table 6. The adult cattle exhibited the highest positive rate of anti-BVDV antibodies (59.72%), exceeding the rates observed in calves (34.55%) and young cattle (42.36%). Overall, these findings provide valuable insights into the epidemiology of BVDV infection, highlighting variations in infection rates among different areas, farm types, cattle purposes, and age groups.

Table 2: Comparative Analysis of E2 Test Strips and Commercial ELISA Kit for Anti-BVDV Detection

E2 test strips	Positive		Negative		Total
	No.	%	No.	%	
Positive (+)	146	96.68	5	3.31	151
Negative (-)	12	11.42	93	88.57	105
Total	158	61.71	98	38.28	256

Table 3: Results obtained from the clinical sample tests conducted at five different areas

Areas	Positive herds/ Checked herds	Herd positive rate (%)	Positive /Total No	No Positive rate (%)
Shihezi	5/5	100	106/243	43.62
Shawan	7/9	77.78	237/464	51.07
Manasi	5/5	100	186/276	67.40
Kuitun	8/9	88.89	365/643	56.76
Ili	8/8	100	206/409	50.36
Total	33/36	91.67	1100/2035	54.05

Table 4: Anti-BVDV test outcomes in bovine serum samples sourced from various origins

Herd source	Positive herds /Checked herds	Herd positive rate (%)	Positive No /Total No	Positive rate (%)
Retail farm	15/18	83.33	83/462	17.97
Large-scale farm	12/12	100	972/1573	61.76
Total	33/36	91.67	1100/2035	54.05

Table 5: Outcomes of the Anti-BVDV antibody tests conducted on bovine serum samples obtained from cattle utilized for diverse purposes

Herd use	Positive		Positive rate (%)
	Number	Total Numbers	
Beef cattle	325	805	40.37
Milk cow	722	1093	66.05
Breeding cattle	53	137	38.69
Total	1100	2035	54.05

Table 6: exhibits the results of the anti-BVDV antibody detection conducted on bovine serum samples derived from cattle across various age groups

Age	Positive		Positive rate (%)
	Number	Total Numbers	
Calves	36	143	25.17
Young cattle	161	380	42.36
Cattle	903	1512	59.72
Total	1100	2035	54.05

DISCUSSION

BVDV has become a widespread threat globally, impacting animal health and production performance, and resulting in substantial economic losses in the global livestock industry (Ran et al. 2019).

Due to the challenges associated with virus isolation, the majority of research concentrates on nucleic acid detection in the 5'-UTR regions for identifying and classifying the virus. This results in a lack of reliable evidence for BVDV genotyping and comprehensive genetic characteristics analysis (Chang et al. 2021; Afify et al. 2022).

Currently, the detection of anti-BVDV antibodies is predominantly accomplished through ELISA. While ELISA offers a straightforward approach, it is time-intensive, requiring approximately 2 hours to complete the experimental procedure. Moreover, it necessitates the use of pipettes and a micro plate reader for accurate measurement of sample optical density (OD). Consequently, specific experimental equipment is indispensable. Additionally, ELISA demands skilled operators to mitigate the risk of contamination, which may

result in false positives, thereby compromising result interpretation.

In contrast to traditional diagnostic methods such as ELISA, colloidal gold strips offer a superior approach for detecting BVDV infections. These strips provide rapid results within a timeframe of 10-15 minutes, making them highly suitable for time-sensitive scenarios. Notably, their visual interpretation feature obviates the need for specialized equipment, enhancing their utility in resource-limited settings. Furthermore, colloidal gold strips facilitate the direct detection of BVDV antigens in whole blood samples, eliminating the serum separation step required by ELISA. This streamlined process not only expedites testing but also reduces assay time significantly, enhancing efficiency and throughput. From a cost-effectiveness perspective, colloidal gold test strip materials are economical, and their production is optimized through the utilization of the BL21 (DE3) E. coli expression system. This approach ensures efficient production of the essential E2 protein, contributing to the affordability and scalability of the diagnostic methodology.

Notably, literature reports suggest that aside from cattle, BVDV can infect various other animals including pigs, sheep, deer, camels, and diverse wild species. However, due to the species specificity of ELISA HRP-labeled antibodies, commercial ELISA kits can solely detect the content of anti-BVDV antibodies within a specific animal species. Colloidal gold strips offer a breakthrough in overcoming this limitation, enabling the detection of anti-BVDV antibodies across a wide range of animal species. This advancement holds significant promise in assisting farms in eradicating BVDV more effectively. By providing a rapid and accessible means of detecting BVDV antibodies, colloidal gold strips empower farmers with the tools needed to implement targeted disease control measures and ultimately contribute to the reduction of BVDV prevalence within animal populations.

The glycoprotein E2 of the BVDV E2 protein plays a crucial role as it exhibits neutralizing activity and contains the primary antigenic determinant of the virus. It possesses strong immunogenicity and serves as the main target for neutralizing antibodies in animals infected with BVDV (Grigera et al. 2000). In natural infections, the immune response primarily focuses on viral proteins E2 and NS2-3, while eliciting a comparatively weaker response against viral proteins Erns and E1. Therefore, the E2 protein is considered the predominant protein for BVDV vaccine development and diagnostic applications (Mv 1995).

Various systems are currently available for exogenous gene expression, including *E. coli* expression systems, mammalian expression systems (Thomas et al. 2009), Drosophila melanogaster systems (Robiolo and Schauer 2007), baculovirus expression systems (Pecora et al. 2012; Zoth and Taboga 2006), *Saccharomyces cerevisiae* yeast systems (Patterson et al. 2012), alfalfa plants (Aguirreburualde et al. 2013), yeast Pichia pastoris expression systems (Behera et al. 2015) and insect cell expression systems. However, the E. coli expression system remains the preferred choice for expressing foreign proteins due to its well-defined genetic background, rapid reproduction, cost-effectiveness, high expression levels, and ease of operation (Lee and Lee 2003).

Bovine Viral Diarrhea (BVD) is a significant disease that poses a threat to animal husbandry. Currently, effective treatments for BVD are limited, leading to substantial economic losses in the livestock industry. The economic impact per cow can range from $40 to $95 due to persistent infection or reproductive failures caused by BVDV toxicity. In cases of highly virulent BVDV infection or secondary infections with other pathogens, the economic loss per cow can exceed $400 (Houe 2003).

The BVDV pathogen undergoes mutations influenced by environmental factors such as climate and seasons, resulting in more complex clinical symptoms and pathogenesis (Zhao et al. 2009). Some strains of BVDV can even cause acute death with mortality rates ranging from 17 to 32% (Ridpath 2013). Consequently, BVD remains a significant hidden danger in the cattle industry, posing greater challenges for prevention and treatment compared to previous times (Mv 1995).

In recent times, BVDV infection and associated diseases have become widespread in China, leading to significant economic losses in the animal husbandry sector (Liu et al. 2024). Up to now, there are mainly BVDV-1 species in China, while the BVDV-2 species are rarely found (Yang et al. 2022).

Since there is currently no commercial BVDV vaccine available in China, the antibody levels in cattle herds can serve as indicators of BVDV infection status. Utilizing E2 test strips, a total of 2035 clinical samples were gathered from 36 cattle farms located in various regions across Xinjiang, including Shihezi, Kuitun, Manas, Yili, and Shawan. The antibody-positive rate among the sampled herds was remarkably high, with 33/36 herds testing positive, translating to a prevalence rate of 91.67%. At the individual level, the positive rate stood at 54.05%, indicating that 1100/2035 tested individuals were positive for BVDV infection. BVDV infections were detected in dairy cows, beef cattle, and breeding cattle, indicating varying levels of BVDV infection across different cattle breeds in Xinjiang. Specifically, dairy cows exhibited a high positive rate of 66.05% (722 out of 1093), while breeding cattle had a lower positive rate of 38.69% (137 out of 353).

In general, the antibody-positive rates in cattle farms range from 60% to 85%, with a persistent infection rate of 1% to 2% (Wang et al. 2010). Among the 36 herds monitored in this survey, 20 sites exhibited antibody-positive rates above 60%, with three fields having rates exceeding 85%. Furthermore, the antibody-positive rate was higher in adult bovines with 59.72% (903/1512) testing positive for BVDV antibodies. In contrast, the positive rate among calves was lower, at 25.17% (36/143). This discrepancy suggests that adult bovines may be more susceptible to BVDV infection compared to young cattle and calves in the sampled population. This suggests that most positive herds may have persistent cattle infections. The study observed a notable difference in BVDV infection rates between large-scale cattle farms and retail farms. Specifically, the infection rate at large-scale cattle farms were 100%, with all 12 farms sampled testing positive for BVDV. The positive rate among the cattle on these large-scale farms was 61.76%, with 972 out of 1573 individuals testing positive.

In comparison, the infection rate at retail farms was slightly lower at 83.33%, with 83 out of 462 farms testing positive for BVDV. This discrepancy underscores the significant impact of factors such as stocking density and environmental sanitation on BVDV infection. The higher infection rate in large-scale cattle farms suggests that these factors may play a crucial role in the transmission and spread of BVDV within the cattle population. It is crucial to prioritize BVD prevention and control measures when expanding production scale. Improving environmental sanitation, controlling stocking density, and enhancing the overall health of cattle are effective strategies to prevent BVD outbreaks. These findings imply that many farms have infected animals (Evans et al. 2019). In the present scenario, the most effective approach to controlling BVDV transmission involves combining vaccination with the detection and elimination of persistently infected cattle from healthy groups (He et al. 2022). Due to the continuous mutation of BVDV strains, existing BVDV vaccines do not offer complete protection against all BVDV1 and BVDV2 strains (Wang et al. 2014). To address the challenge of BVDV control and eradication in large-scale cattle farms, epidemiological research plays a vital role in providing crucial information for disease prevention and control. Therefore, the development of colloidal gold test strips using E2 protein offers good specificity, high sensitivity, and simple and quick operation, providing technical support for BVDV eradication within herds and serving as a direct basis for serological investigations.

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Conflict of interest

The authors declare that they have no financial or personal relationships with individuals or organizations that could potentially influence or bias the content of this paper.

Author's contribution

Adnan Ali write the paper, Sheng Jinliang write analyse and proofread the paper, Xiao Shengzhong proofread the paper, Li Yan make images and graphs, Zhang Yanhong make images and graphs, Yang Yan perform lab work, Hao Jingxiu perform lab work, Shi Feng perform lab work.

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