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Short Communication

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A novel detection method of *Cryptosporidium parvum* infection in cattle based on *Cryptosporidium parvum virus* 1

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Abstract

Cryptosporidium parvum is an important zoonotic parasite that causes significant economic loss in the animal husbandry industry, especially the cattle industry. As there is no specific vaccine or drug against *Cryptosporidium*, a rapid and accurate method for the detection of *C. parvum* is of great significance. In this study, colloidal gold strips were developed based on *Cryptosporidium parvum virus* 1 (CSpV1) for the detection of *C. parvum* infection in cattle fecal samples. The colloidal gold solution was prepared by reducing trisodium citrate and the CSpV1 #5 monoclonal antibody was labeled with colloidal gold. A polyclonal antibody against the CSpV1 capsid protein and an anti-mouse IgG antibody were coated on the colloidal gold strips for use in the test and control lines, respectively. Our results showed that the detection sensitivity in fecal samples was up to a 1:64 dilution. There was no cross-reaction with *Cryptosporidium andersoni* or *Giardia* in the fecal samples. The different preservation conditions (room temperature, 4°C, and 37°C) and preservation time (7, 30, 60, and 90 days) were analyzed. The data showed that the strips could be preserved for 90 days at 4°C and for 60 days at room temperature or 37°C. The colloidal gold strips were used to detect the samples of 120 clinical fecal in Changchun, China. The results indicated that the rate of a positive test was 5% (6/120). This study provides a rapid and accurate method for detecting *C. parvum* infection in cattle and humans.

Key words: *Cryptosporidium parvum virus* 1, *Cryptosporidium parvum*, colloidal gold strip, detection method

Introduction

Cryptosporidium is an important parasite [1] that can cause severe diarrhea in healthy patients and long-term and life-threatening infections in immunocompromised patients (such as AIDS patients) [2]. *Cryptosporidium* can infect more than 260 host species and cause significant loss in the animal husbandry industry each year. *Cryptosporidium* is ranked fifth among the 24 most important foodborne parasites listed by the UN Food and Agriculture Organization (FAO) and the World Health Organization (WHO). This organism is an important zoonotic parasite that has been neglected in recent years.

There are many species of *Cryptosporidium* that can infect cattle, including mainly *Cryptosporidium andersoni*, *Cryptosporidium parvum*, *Cryptosporidium bovis*, and *Cryptosporidium ryanae* [3]. Of these, *C. parvum* has a wide range of hosts and is the most harmful to human health [4]. The cattle industry, with its rapid

development, has become the main source of human cryptosporidiosis [5,6]. Additionally, there are no specific vaccines or drugs against *Cryptosporidium* [7], and therefore, a rapid and accurate method for the detection of *C. parvum* is of great significance to both animal husbandry and human health.

C. parvum detection methods mainly include etiological, molecular biology, and immunological detection methods. Detection of *Cryptosporidium* oocysts in feces or water samples by microscopy is the gold standard for determining the presence of this organism [8]. However, this method has low sensitivity and specificity and is not suitable for detecting the large number of samples [9]. Molecular biology detection methods mainly include polymerase chain reaction (PCR), real-time PCR, cleaved amplified polymorphic sequences (CAPs), and loop-mediated isothermal amplification (LAMP). These techniques are mainly used to detect the oocysts of *C. parvum* in water or feces, but they are not suitable for rapid detection in the clinic due to the long detection time. Most of the immunological detection methods involve the detection of antibodies in serum samples, such as indirect immunofluorescence (IFA) and enzyme linked immunosorbent assay (ELISA); however, these methods are unable to determine present infection. Considering the existed methods with various advantages and disadvantages in clinical application, a specific, sensitive and rapid detection method is very important for the prevention of *Cryptosporidium* infection in the future.

In recent years, it has been found that a viral symbiont exists in the oocysts of *C. parvum* [10,11], which was named as *Cryptosporidium parvum virus* 1 (CSpV1). CSpV1 is a dsRNA virus consisting of L-dsRNA and S-dsRNA. It has a capsid protein on the surface of oocysts, which is encoded by S-dsRNA and has a protein molecular weight of 37 kDa. The dsRNA virus in the present study was found in all strains of *C. parvum* [12–15]. There are approximately 2000 virus particles in each *C. parvum* oocyst; therefore, the sensitivity of detection of CSpV1 is 2000 times higher than that of other oocyst-based detection markers of *C. parvum*. CSpV1 is also stable in oocysts and can be used as an important detection indicator of *C. parvum* infection in cattle [16]. Therefore, in this study, we developed CSpV1-based colloidal gold strips for the detection of *C. parvum*. This method has the advantages of rapid, specific and sensitive in the clinic and provides a way to dynamically monitor *C. parvum* in the future.

Materials and Methods

Expression and purification of CSpV1 capsid protein

Based on the nucleotide sequence of the CSpV1 capsid protein in GenBank (KY884721.1), forward and reverse primers were designed with *Bam*HI and *Sal*I restriction enzyme cut sites (forward primer: 5′-ggatccATGATTACAAGTTTTGAATCAATAGAG-3′; reverse primer: 5′-gtcgacCTAGTGGGAGCGATCTGT-3′). The gene was amplified by PCR using *C. parvum* cDNA (preserved in our laboratory) as template. The PCR product was ligated into the expression vector pET-28a (Solarbio, Beijing, China) to construct a recombinant plasmid named pET-28a-CSpV1-S. The expression vector pET-28a-CSpV1-S was transformed into *Escherichia coli* BL21 (DE3) cells. Monoclonies were selected and cultured in LB medium, when the OD value of the bacterial liquid reached 0.6, the recombinant protein was expressed under 1.0 mM IPTG (Solarbio) induction for 5 h at 37°C. Then the induced bacterial liquid was centrifuged for 10 min at 10,000 *g* and resuspended in PBS. After ultrasonification, the induced protein sample was obtained. The protein was purified using a Ni-NTA purification system (Sangon Biotech, Shanghai, China) according to the manufacturer's protocol.

Identification of CSpV1 capsid protein

The induced protein sample was centrifugated for 10 min at 8,000 *g*, the supernatant was collected, and the sediment was resuspended in PBS and collected. The induced protein sample, an uninduced sample, a pET-28a empty vector control sample a sediment and a supernatant sample were mixed with 6×Protein Buffer (TransGen Biotech, Beijing, China) and boiled for 10 min. Thirty micrograms of each protein sample were separated on a 15% SDS-PAGE gel, then transferred to PVDF membrane (Millipore, Bedford, USA). The membrane was blocked with 5% defatted milk, and incubated with *C. parvum*-infected bovine serum (1:100; preserved in our laboratory) as the primary antibody, followed by incubation with HRP-conjugated mouse anti-bovine IgG secondary antibody (Solarbio). The membranes were visualized using an ECL Western Blot Detection System (Clinx Science Instruments Co, Shanghai, China).

Preparation of rabbit anti-CSpV1 capsid protein polyclonal antibody

A total of 500 μg of purified CSpV1 capsid protein was added to an equal volume of Freund's complete adjuvant (Sigma, St Louis, USA) for emulsification. The emulsion was then used to subcutaneously immunize 2-month-old rabbits. After 15 days, a second immunization was performed as the first immunization. After 7 days, a third immunization was performed using the same method described above. After another 7 days, serum was collected and stored at −20°C, the serum titers were measured by indirect ELISA. Finally, polyclonal antibody was purified by a protein A column (TransGen Biotech). The specificity of antibody was tested by western blot analysis, and the whole proteins of *C. andersoni* oocysts, *C. parvum* oocysts, and *Giardia* were extracted and selected as antigens, rabbit anti-CSpV1 antibody was used as the primary antibody, the second antibody was HRP-labeled goat anti-rabbit IgG (ZSGB-Bio, Beijing, China).

Indirect ELISA

The purified CSpV1 capsid protein was coated in a 96-well plate in an amount of 1 μg/well, and the plates were incubated at 37°C for 1 h. Then the plate was washed five times with PBST buffer (0.002 M PBS containing 0.05% Tween-20). The blocking buffer (PBST containing 5% defatted milk; 100 μl) was added to 96-well plate and incubated at 37°C for 2 h. One hundred microliters of the test serum and negative serum (diluted from 1:1000 to 1:204,800) was added to each well and incubated at 37°C for 2 h. After washing, HRP-labeled goat anti-rabbit IgG (1:5000; ZSGB-Bio) was added. After that, TMB ELISA substrate (TransGen Biotech) was added to each well and incubated for 15 min. Color development reaction was stopped by addition of 1 M H_2SO_4. The OD values were measured at a wavelength of 450 nm.

Colloidal gold solution preparation

Ultrapure water (99 ml) was added to a round-bottom flask and placed on a heated magnetic stirrer, with the heating switch turned on. After the stir bar was covered with many bubbles, 1 ml of chloroauric acid solution was added and the speed of the rotor was adjusted. After the first bubble appeared, 1 ml of trisodium citrate solution was quickly added. The solution was heated for 5 min once the color gradually changed to burgundy. Finally, water was added to adjust the volume of the solution to 100 ml. The colloidal gold solution was dropped onto nickel mesh, observed by transmission

electron microscopy (TEM) (HITACHI, Tokyo, Japan), and stored at 4°C in the dark.

Optimal pH and amount of monoclonal antibody

The prepared colloidal gold solution (1 ml) was aliquoted into five centrifuge tubes, and 1, 2, 3, 4, or 5 µl of 0.2 M K_2CO_3 was added to each tube; each sample was assayed in triplicate. The solutions were mixed well, and 30 µg of anti-CSpV1 capsid protein #5 monoclonal antibody (prepared previously in our laboratory and stored at –20°C; 1:160,000 dilution) was added. The solution was sufficiently mixed and stored at room temperature for 30 min. Then, 100 ml of 10% NaCl was added to each tube, and the tubes were incubated for 2 h at room temperature. The optimal pH of the colloidal gold solution was determined based on the color change.

The colloidal gold solution (1 ml) was added to six centrifuge tubes. After the solution was adjusted to the optimal pH, 0, 5, 10, 15, 20, or 25 µg of anti-CSpV1 capsid protein #5 monoclonal antibody was added to each tube, and each amount of mAb was assayed in triplicate. The tubes were mixed well and stored at room temperature for 30 min. Then, 100 ml of 10% NaCl was added to each tube, and the tubes were incubated for 2 h. The optimal amount of monoclonal antibody was determined based on the color change. The solution was dropped onto nickel mesh and observed by TEM.

Preparation of colloidal gold strips

After the colloidal gold solution was adjusted to the optimal pH, and the optimal amount of monoclonal antibody was added, 10% BSA (in 0.02 M Tris-HCl) was added (1:10), and the solution was centrifuged at 13,000 *g* for 30 min at 4°C. The sediment was resuspended in a resuspension solution (1:10; Hui Bai, Shenyang, China). The solution was evenly placed onto the released gold–mAb conjugation pad. The absorption pad, nitrocellulose membrane, released gold–mAb conjugation pad, and sample application pad were assembled in order into a PVC pad and cut into individual strips (4.0 mm/strip) with a paper cutter.

Colloidal gold strip test criteria

Rabbit anti-CSpV1 capsid protein polyclonal antibody (1 µl) was added to the test line (T), and goat anti-mouse IgG antibody (ZSGB-Bio) was added to the control line (C). A sample was determined to be positive when a color change occurred on both the C and T lines. If a color change occurred on the C line but not on the T line, the sample was determined to be negative.

Optimal antibody concentration on the test line (T) and control line (C)

The polyclonal antibody was diluted from 2 mg/ml to 0.25 mg/ml and added to the T line, while the control line was coated with 1 µl of 1 mg/ml goat anti-mouse IgG antibody. Then, a sample of *C. parvum* infection was used, the optimal antibody concentration was determined based on the color of the T line.

The goat anti-mouse IgG antibody was diluted from 1 mg/ml to 0.125 mg/ml and added to the C line, while the test line was coated with 1 µl of 0.5 mg/ml polyclonal antibody. Then, a *C. parvum*-infected sample was used, and the optimal concentration was determined based on the color change of the C line.

Performance tests

The *C. parvum*-negative samples and fecal samples of *C. parvum, C. andersoni* and *Giardia* infection were detected by colloidal gold strips and microscopic method, each sample was tested three times. The specificity of the method was determined based on these test results.

A sample positive for *C. parvum* infection (~1000 oocysts per gram of sample) was diluted from 1:2 to 1:128 for detection by the colloidal gold strips, and each dilution was measured three times. The sensitivity result was determined based on the color change of the test line, and compared with microscopic method.

Three batches of colloidal gold strips were selected to detect negative and positive samples of *C. parvum* infection, and there were two replicates for each batch. The reproducibility of this method was determined based on the color of the test line.

In order to test the stability of this method, the colloidal gold strips were stored under different storage conditions (room

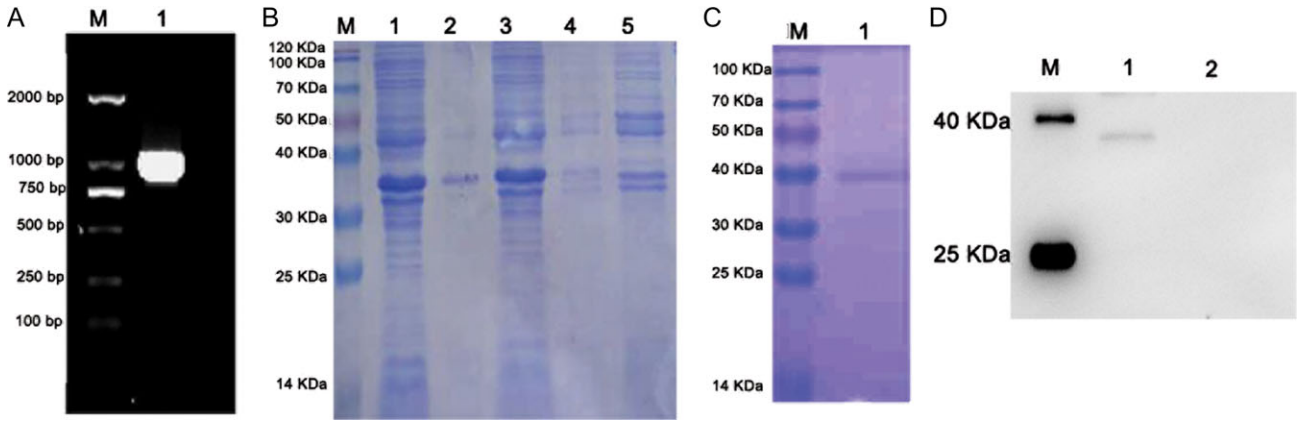

Figure 1. Expression of the CSpV1 capsid protein (A) PCR analysis of CSpV1 capsid protein using 1% agarose gel electrophoresis. Lane M is the DNA Marker (DL2000), and lane 1 is the CSpV1 capsid protein PCR product. (B) SDS-PAGE analysis of recombinant protein. Lane M is the protein molecular weight marker; lane 1 is the protein supernatant sample; lane 2 is the protein precipitate sample; lane 3 is the induced bacterial sample; lane 4 is the uninduced bacterial sample; and lane 5 is the empty pET-28a vector bacterial sample. (C) SDS-PAGE analysis of purified recombinant protein. Lane M is the protein molecular weight marker, and lane 1 is purified pET-28a-CSpV1-S recombinant protein. (D) Western blot analysis of purified pET-28a-CSpV1-S recombinant protein. Lane M is the protein molecular weight marker; lane 1 is pET-28a-CSpV1-S recombinant protein; and lane 2 is empty bacterial pET-28a vector.

Table 1. The results of rabbit anti-CSpV1 capsid protein polyclonal antibody titers

	Serum dilution											
	1000	2000	4000	8000	16000	32000	64000	128000	**256000**	512000	1024000	2048000
P	0.838	0.793	0.715	0.585	0.514	0.413	0.292	0.216	**0.171**	0.128	0.106	0.100
N	0.132	0.118	0.101	0.071	0.091	0.078	0.079	0.079	**0.081**	0.089	0.082	0.094
P/N	6.348	6.720	7.079	8.239	5.648	5.294	3.696	2.734	**2.111**	1.438	1.293	1.064

temperature, 4°C, and 37°C) and for different storage periods (7, 30, 60, and 90 days), and then the stability was determined according to the detection results of *C. parvum* infectionfaecal samples.

Clinical samples test

A total of 120 clinical samples from Changchun, China, were tested using the prepared colloidal gold strips, and the test results were compared with those of the commercial ELISA kit (MEIMIAN, Yancheng, China) and PCR method (primers were designed according to 18 S rRNA of *C. parvum*, GenBank: L16996.1, forward: 5′-GCCTGAGAAACGGCTACCA-3′, reverse: 5′-CTCCCCCCAGAA CCCAAAG-3′, the PCR product is 696 bp). The results of each method were analyzed.

Results

Successful expression of the CSpV1 protein

The CSpV1 PCR product was analyzed by 1% agarose gel electrophoresis, and the result showed a clear band at 960 bp (**Fig. 1A**). Sequencing results were compared with the gene sequence published in GenBank (KY884721.1), and the similarity was 99%.

The recombinant protein was analyzed using SDS-PAGE and western blot analysis. As shown in **Fig. 1B**, the induced protein sample showed a clear target band at 37 kDa, which indicated that the target protein was successfully expressed. The CSpV1 capsid protein was purified by a Ni-NTA purification system and analyzed by SDS-PAGE. As shown in **Fig. 1C**, the purified protein sample showed a single band at 37 kDa. As shown in **Fig. 1D**, the purified CSpV1 capsid protein was recognized by serum from a *C. parvum*-infection cattle, and the target band appeared at 37 kDa, while no band was detected for the empty vector.

Detection of polyclonal antibody titer using ELISA

Indirect ELISA was used to detect anti-CSpV1 capsid protein polyclonal antibody. The results indicated that the polyclonal antibody titer was 256,000 (**Table 1**). As shown in **Fig. 2**, the whole proteins of *C. andersoni* oocysts and *Giardia* could not be recognized by anti-CSpV1 antibody, and there was a single band in the lane of *C. parvum* oocyst whole proteins, suggesting that the antibody is highly specific.

TEM observations

Observation of the colloidal gold particles by TEM revealed that the colloidal gold particles were uniformly dispersed and that there was no particle aggregation (**Fig. 3A**). The particles of the colloidal gold–mAb conjugate were observed by TEM as well. As shown in **Fig. 3B**, there was a significant halo around the colloidal gold particles, demonstrating successful labeling with the monoclonal antibody.

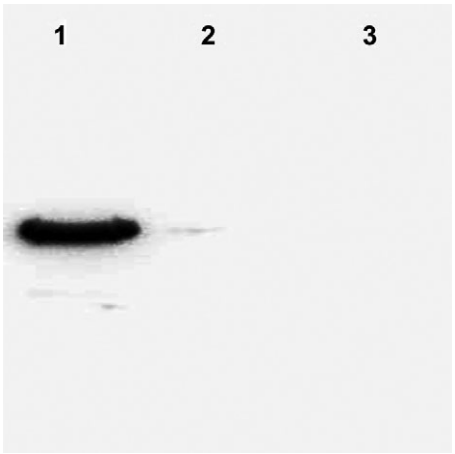

Figure 2. Specificity of polyclonal antibody Lane 1 is the whole protein sample of *C. parvum* oocysts; lane 2 is the whole protein sample of *C. andersoni* oocysts; lane 3 is the whole protein sample of *Giardia*.

Optimization of colloidal gold strips

The optimal pH of the colloidal gold solution was determined based on the amount of 0.2 M K_2CO_3 added. As shown in **Fig. 4A**, when 4 μl of 0.2 M K_2CO_3 was added, the color of the colloidal gold solution changed from blue to red, and colloidal gold particles did not aggregate or precipitate. Therefore, the optimal pH of colloidal gold solution was 7.5.

As shown in **Fig. 4B**, the color of the colloidal gold solution changed from blue to red in the second centrifuge tube (5 μg), and on this basis, we used 6 μg antibody (20% more than 5 μg) as the optimal amount of anti-CSpV1 # 5 monoclonal antibody.

When the anti-CSpV1 capsid protein polyclonal antibody was diluted to 0.5 mg/ml, the T line was still clear and the amount of antibody used was minimal. Therefore, the optimal coating concentration of the T line was 0.5 mg/ml (**Fig. 5A**).

As shown in **Fig. 5B**, when the goat anti-mouse IgG antibody was diluted to 0.25 mg/ml, the color of the C line was obvious, and the amount of antibody used was minimal. Thus, the optimal coating concentration of the control line (C) was 0.25 mg/ml.

Performance test results

The specificity test showed that two clear red lines were observed in the T and C lines for the detection of *C. parvum* infection in fecal samples, and there was no cross-reaction with fecal samples of *C. andersoni* or *Giardia* infection (**Fig. 6A**). These results are in agreement with microscopic detection method. As shown in **Fig. 6B**, there was a clear and obvious color change in the T line when the sample was diluted to 1:64, and the C line only appeared when the dilution reached 1:128. Therefore, the sensitivity of colloidal gold strips was 1:64 (~16 oocysts per gram of sample). Compared with the

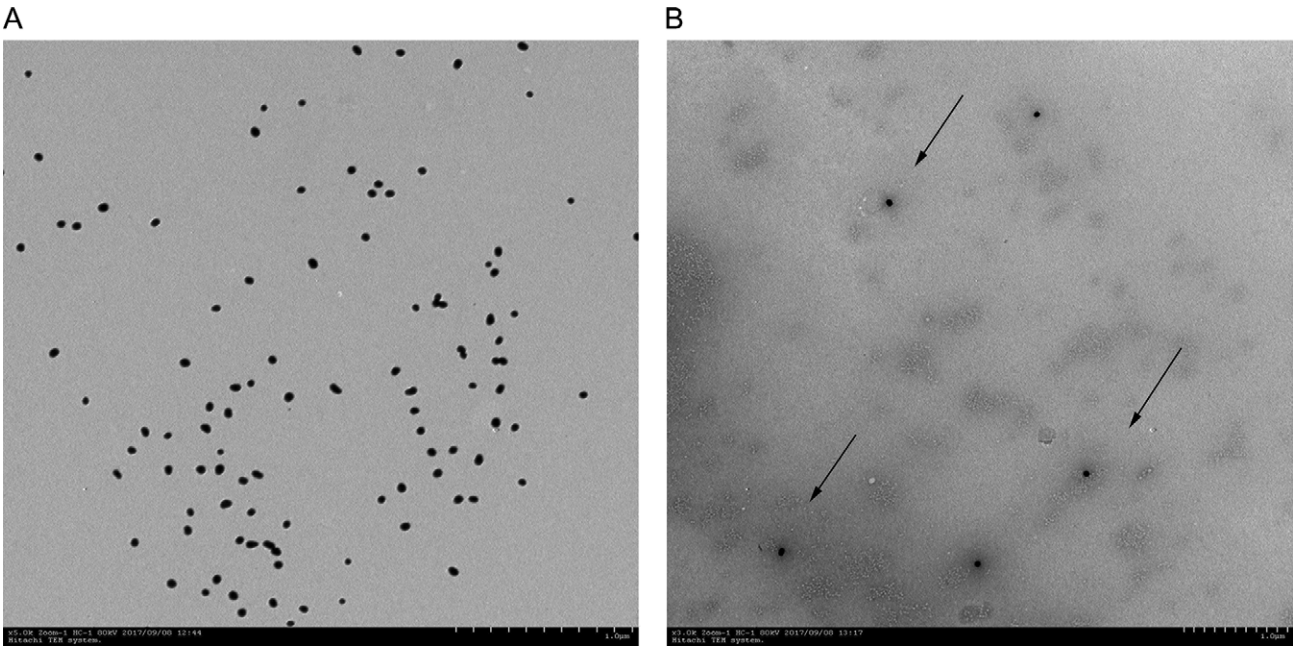

Figure 3. Observations of colloidal gold particles and colloidal gold–mAb conjugation (A) TEM images of colloidal gold particles. (B) TEM images of antibody-colloidal gold conjugates.

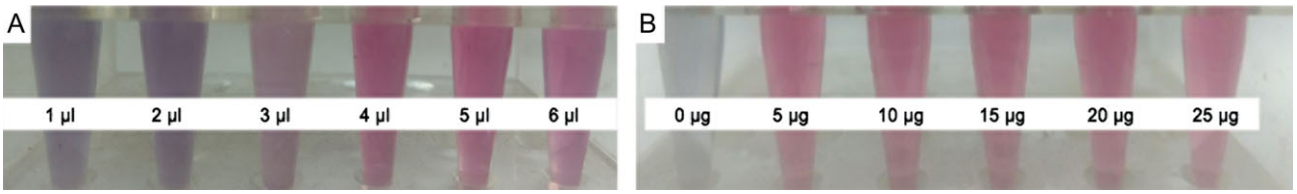

Figure 4. Optimization of pH and amount of monoclonal antibody The optimal pH and amount of monoclonal antibody were determined based on the color change of colloidal gold solution. (A) The optimal pH of colloidal gold solution. (B) The optimal amount of monoclonal antibody.

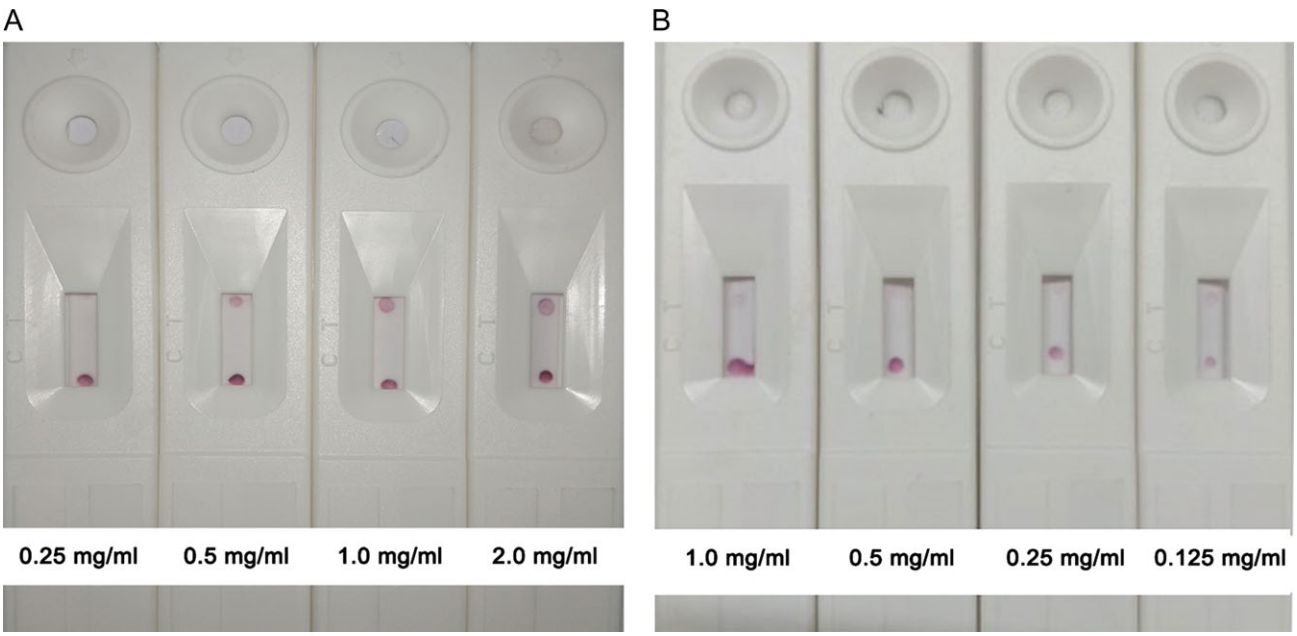

Figure 5. Optimal coating concentrations of the T and C lines The optimal coating concentration was selected based on the clarity of the color and the minimum amount of antibody required. (A) The optimal coating concentration for the T line. (B) The optimal coating concentration for the C line.

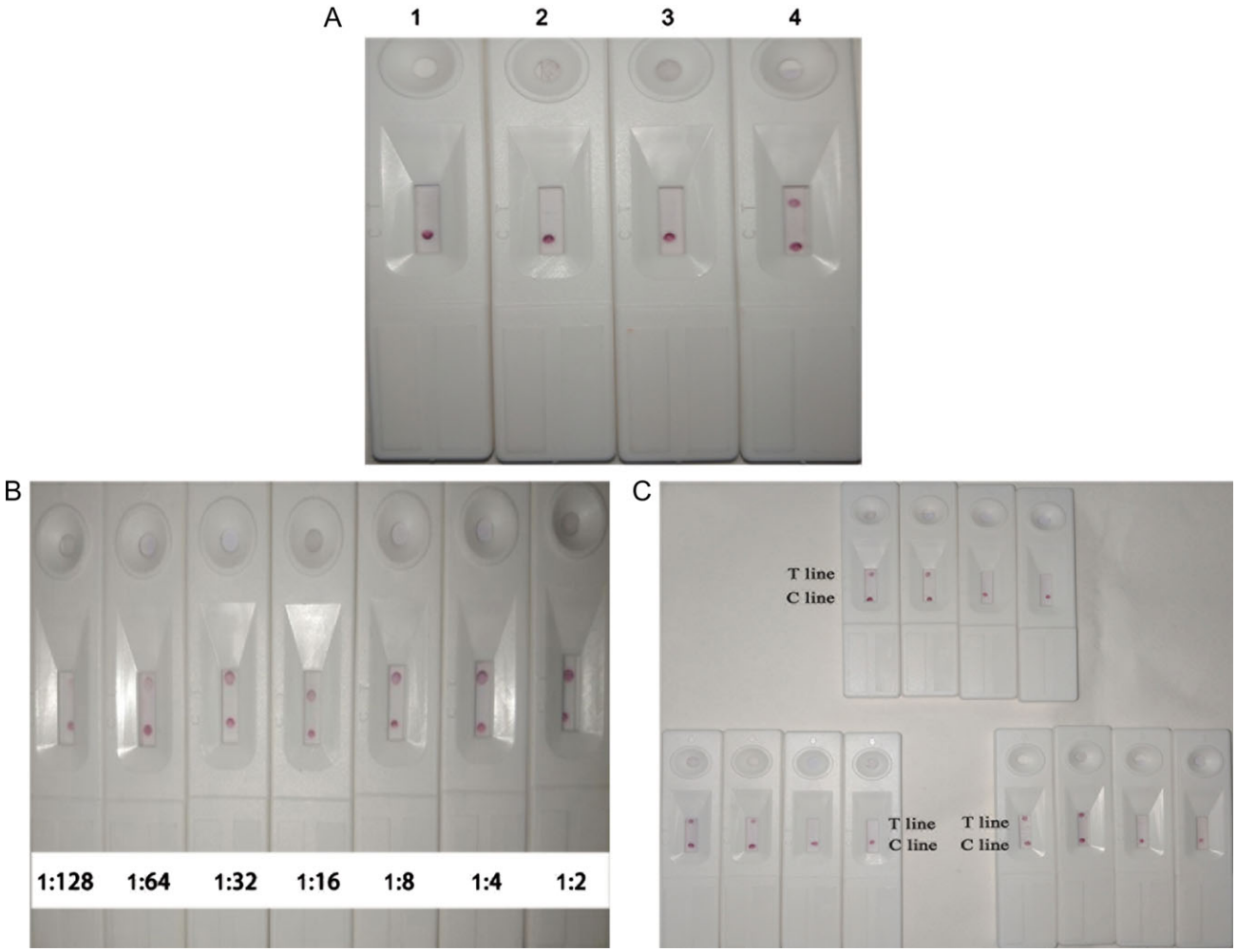

Figure 6. Performance test results of colloidal gold test strip (A) For the specificity test, strips 1–4 were used to analyze a *C. andersoni*-infected sample, *Giardia*-infected sample, *C. parvum*-negative sample and *C. parvum*-infected sample, respectively. (B) For the sensitivity test, when the sample was diluted to 1:128, the T line was completely invisible, indicating that the sensitivity is up to 1:64. (C) For the reproducibility test, positive and negative *C. parvum* samples were tested using three batches of test strips. The results were consistent across batches.

Table 2. The results of the stability test

	7 days	30 days	60 days	90 days
Room temperature	+++	++	+	–
4°C	+++	+++	+++	++
37°C	+++	++	+	–

'+++' indicated that the T line was very clear; '++' indicated that the T line was relatively clear; '+' indicated that the T line was lighter in color; '–' indicated that the T line was invisible.

microscopic detection method (the sensitivity was 1.4×10^6 per gram of sample), it has a higher sensitivity for detection of *C. parvum* in fecal samples. Reproducibility tests were performed on the same and different batches of test strips for the detection of *C. parvum* infection in positive and negative samples. The results showed that the reproducibility of the test strips was good (**Fig. 6**C). The results of the stability test are shown in **Table 2**. The colloidal gold strips could be stored at 4°C for at least 90 days and at room temperature or 37°C for 60 days.

Clinical samples testing

We used the test strips to detect 120 cattle fecal samples in Changchun, China. As shown in **Fig. 7**, the positive sample rate of the colloidal gold strips was 5% (6/120). Identified positive samples included No. 7, No. 32, No. 34, No. 52, No. 53., and No. 54. The positive samples identified by the colloidal gold strips were consistent with those identified by commercial ELISA kits and PCR tests.

Discussion

In 1971, detection methods based on colloidal gold technology were used in the field of immunology for the first time [17]. Colloidal gold has the unique advantage because it can produce a color reaction and bind with antigens, antibodies, and other biomacromolecules. At present, there are many detection methods for pathogens; however, they have many shortcomings in clinical application, such as long detection time, complicated operations, difficult preservation requirements, and the need of expensive detection instruments. Colloidal gold technology can overcome these shortcomings and has been widely used in the detection of pathogens *in vitro*. Niu *et al.*

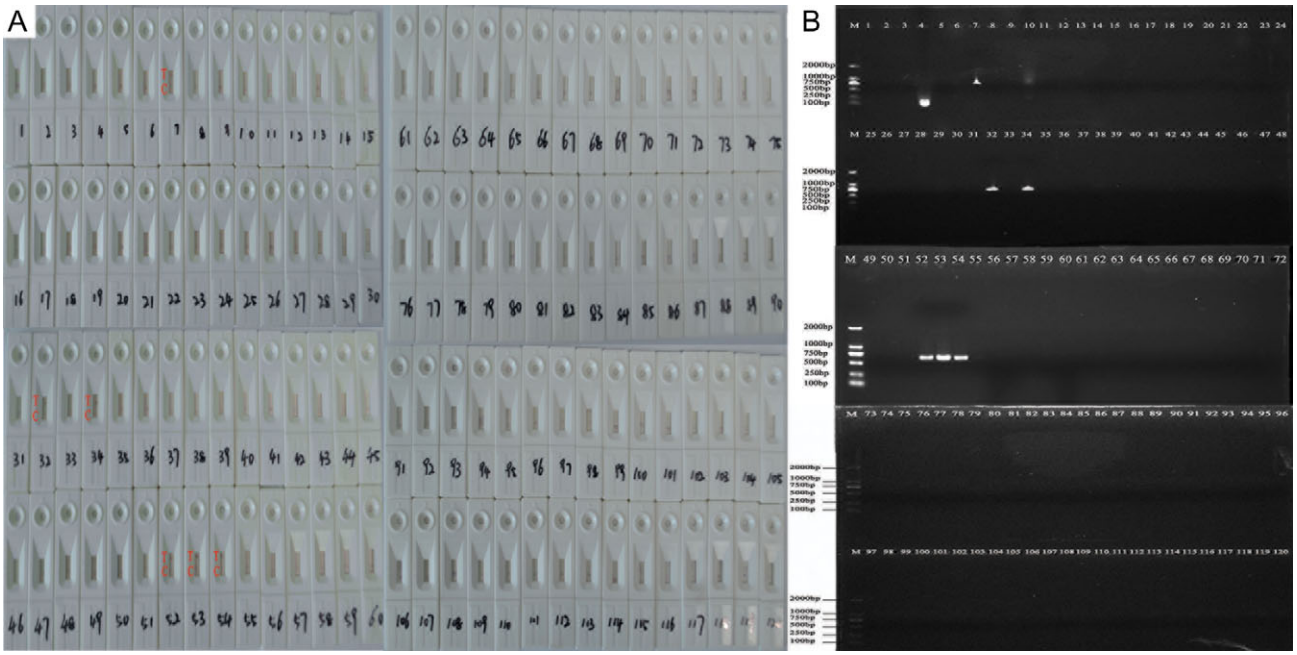

Figure 7. Clinical sample test using different methods A total of 120 clinical samples were tested by the colloidal gold strips and PCR method. A color change occurred on both the C and T line when the samples (included No. 7, No. 32, No. 34, No. 52, No. 53, and No. 54) were detected by the test strips. The results of PCR method were analyzed by 1% agarose gel electrophoresis. The positive samples detected by the test strips were consistent with those detected by PCR method. (A) The results of colloidal gold strips method. (B) The results of PCR method.

[18] developed an immunocolloidal gold test strip for the detection of *Staphylococcus aureus*, which was applied for the rapid detection of *S. aureus* in food with a detection rate of 98.7%. Omidfar *et al.* [19] prepared an immunocolloidal gold strip for the detection of human serum albumin in urine based on HSA antibodies. They were used to test 40 clinical urine samples, and compared with immunoturbidimetric methods, the colloidal gold tests strips were more sensitive, specific, and accurate.

In this study, a colloidal gold solution was prepared by the reduction of trisodium citrate. The amount of trisodium citrate was inversely proportional to the colloidal gold particle size, which determined the stability of the colloidal gold solution and the sensitivity of the method [20]. Therefore, we prepared colloidal gold particles with a size of 40 nm by adjusting the amount of trisodium citrate. The colloidal gold solution can be stored at 4°C for more than one month, meeting the requirements of subsequent experiments.

It has been reported that viruses are found in *C. hominis*, *C. felis*, *C. meleagridis*, and *C. parvum* but not in other species of *Cryptosporidium*. The virus that exists in *C. parvum* was named *Cryptosporidium parvum virus* 1 (CSpV1), and there are approximately 500 viral particles in each sporozoite of *C. parvum*. One oocyst contains four sporozoites, so the detection sensitivity of *C. parvum* infection based on CSpV1 is increased by more than 2000 times. Kniel *et al.* [21] showed that when *C. parvum* oocysts are placed at 20°C for more than 3 months, CSpV1 can be detected, and the signal is strong. Therefore, CSpV1 may be an important detection marker of *C. parvum* infection. Most cases of cryptosporidiosis in humans are caused by the pathogens *C. hominis* and *C. parvum*. CSpV1 is highly homologous to the virus that exists in *C. hominis*, so it is also considered to be an important detection marker for *C. hominis* cryptosporidiosis in humans. In 2005, Kniel *et al.* [22] used CSpV1 to develop a dot blot hybridization assay for the detection of *C. parvum* in food. The results showed that this method was more sensitive than methods based on the detection of the total number of oocysts or oocyst surface antigens.

There are many immunological detection methods for *Cryptosporidium*, most of which were developed to detect specific antibodies in serum. For example, Shayan *et al.* [23] used immunoblotting to detect expressed surface antigen proteins in bovine serum samples. The results showed that the rate of positive *C. parvum* infection was 37%. Wang *et al.* [24] developed an indirect ELISA detection method based on the expression of the *CP23* gene in *C. parvum* and applied this method to detect *C. parvum* in serum samples from three cattle farms. The rates of positive *C. parvum* infection were 38.46% (25/65), 47.14% (33/70), and 35.56% (16/45) on the three farms. These methods have high specificity; however, compared with methods for detection in fecal samples, serum samples are inconvenient to collect in the clinic and are unable to discriminate between past and current infections. The same batch of 120 fecal samples from Changchun, China, was tested using our colloidal gold strips, a commercial ELISA kit, and by PCR. Of these samples, six were positive, and the number of positive samples was consistent across these detection methods. In conclusion, the use of colloidal gold strips for the detection *C. parvum* infection is specific, convenient, rapid, and accurate, which is suitable for clinical detection.

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