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RESEARCH ARTICLE

Rapid Detection of *Lawsonia intracellularis* by a Quantitative PCR based on Immunomagnetic Beads

Shunli Yang[1†], Muhammad Umar Zafar Khan[2†], Sumin Pan[1], Xiaoqing Zhang[1], Yupeng Fang[1], Wenyan Gai[3], Jie Zhang[1]* and Yongsheng Liu[1]*

[1]Key Laboratory of Preventive Veterinary Medicine of Hebei, College of Animal Science and Technology, Hebei Normal University of Science and Technology (HNUST), Qinhuangdao, Hebei, 066004, China; [2]Institute of Microbiology, Faculty of Veterinary Science, University of Agriculture Faisalabad, Faisalabad, 38040, Pakistan; [3]Department of Pet Technology, Shandong Vocational Animal Science and Veterinary College, Weifang, Shandong, 261061, China
[†]Equally contributed to this work
*Corresponding author: liuys4031@hevttc.edu.cn (Yongsheng Liu); zhangj4032@hevttc.edu.cn (Jie Zhang)

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ABSTRACT

Immunomagnetic nanobeads (IMNBs) have been widely used to isolate and detect microorganisms. In this study, recombinantly expressed outer membrane proteins LI0902 and LI1024 of *Lawsonia intracellularis* (*L. intracellularis*) were used to yield polyclonal antibodies. IMNBs functionalized with anti-LI0902 polyclonal antibodies display the capture activity to *L. intracellularis* that was indicated by immunofluorescence assay based on anti-LI1024 polyclonal antibody. One-step quantitative PCR (qPCR) target of the aspartate ammonia-lyase gene based on IMNBs was developed and evaluated using several pathogens associated with pig diarrheal diseases and clinical faecal samples. The results showed that IMNBs had good specificity and sensitivity, no cross-reactivity with other pathogens, and the detection limit was as low as 3.6×10^2 copies/mL. Meanwhile, IMNBs showed good capture efficiency, 4.21×10^5 copies of *L. intracellularis* could be captured by 1 µg of IMNBs. Moreover, *L. intracellularis* in faecal samples were successfully detected by the qPCR combined with IMNBs. The results demonstrate that this method is promising in rapidly and effectively detecting *L. intracellularis* in clinical faecal samples.

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INTRODUCTION

Lawsonia intracellularis (*L. intracellularis*), an obligate intracellular curved Gram-negative bacillus, is the pathogen of proliferative enteropathy in a variety of domestic animals (Bengtsson *et al*., 2020). Pig populations are infected by different pathogens including viruses, bacteria, and parasites (Chen *et al.*, 2022; Guo *et al*., 2022). Pigs are the most commonly affected domestic animals, which can suffer from this bacterial infection that affects their enterocytes in the small and large intestines (Collins and Collins, 2024). The typical pathological features of *L. intracellularis* infection are proliferation of the intestinal mucosal layer and chronic intestinal inflammation, known as porcine proliferative enteropathy (PPE) with typical clinical manifestations including diarrhea, hemorrhagic diarrhea, decreased food conversion, and reduced growth rate. Thus, PPE imposes huge economic losses to pig farmers (Lawson and Gebhart, 2000). Although commercial vaccines have been used widely, *L. intracellularis* can still be found in animals worldwide (Bohlin *et al.*, 2019; Barbosa *et al.*, 2023). Therefore, rapid and accurate diagnosis of *L. intracellularis* will help make efficient treatment procedures and prevention programs.

L. intracellularis infection in pigs was reported first in 1931. Since then, the detection techniques for *L. intracellularis* have been developed and applied in veterinary clinical diagnostics and research, such as enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) tests. A specific microaerophilic environment and target cells are obligatory for the *in vitro* culture of *L. intracellularis*

(Vannucci and Gebhart, 2014) which limits the using of the immunofluorescence and immune-peroxidase monolayer assay for laboratory detection of PPE. Thus, PCR methods, including nested PCR, multiplex PCR, conventional PCR, and real-time PCR to target the 16S rRNA (Pedersen *et al*., 2010; Wang *et al*., 2024) and the aspartate ammonia-lyase (*asp*A) gene (Wattanaphansak *et al*., 2010) were usually used for accurate diagnosis of PPE.

Carboxyl superparamagnetic beads have unique properties of being easy to conjugate with recognition units, easy to bind to analytes, and convenient for the isolation operation (Sandhu *et al*., 2010). These one-of-a-kind properties make superparamagnetic beads, functionalized with specific surfaces like antibodies, aptamers, and peptides, hugely popular in biomedical studies and for the detection of pathogens, like viruses (Yang *et al*., 2015a) and bacteria (Kaittanis *et al*., 2007). Antibodies were commonly used to functionalize magnetic beads, which provide high pathogen binding activity and specificity. To exploit the benefits of magnetic beads, a polyclonal antibody specific to outer membrane protein LI0902 was produced and conjugated to the surface of carboxyl-coated superparamagnetic nanobeads, resulting in the formation of immunomagnetic nanobeads (IMNBs). A robust and specific qPCR method for one-step detection of *L. intracellularis* was developed based on IMNBs. The robustness of this method is suggested by its ease of operation, short testing time, and high sensitivity and specificity in detecting *L. intracellularis* from clinical faecal samples.

MATERIALS AND METHODS

Expression of outer membrane protein of *L. intracellularis*: The gene of outer membrane protein LI0902 and partial LI1024 from vaccine strain (Boehringer Ingelheim, USA) was subcloned into the PET-32a (+) vector and expressed in the *E. coli* BL21-Codon-Plus (DE3) strain (TransGen Biotech, China). The expression and purification were carried out as described previously (Yang *et al*., 2018). The concentration was tested by using Nanodrop™ 2000 (Thermo-Fisher, USA) and stored at -80°C.

Preparation of anti-LI0902 and LI1024 polyclonal antibody: New Zealand White Rabbit and BALB/C mice were injected with 50 µg of the recombinant LI1024 and LI0902 (respectively) four times every 2-week intervals. Serum samples were collected after immunization and the antigen-specific antibody titers were monitored. Blood was collected when the rabbit and mouse exhibited more robust antigen-specific antibody responses.

Specific antibodies in serum were monitored by an indirect ELISA. 96-well plates were coated overnight with 10 µg/mL of LI1024 and LI0902 in bicarbonate buffer (pH 9.6, Sigma, USA) respectively. After washing with phosphate-buffered saline with Tween 20 (PBST), 50 µL of the serially diluted sera were added to the well and incubated for 1h at 37°C. The reaction was developed with horseradish peroxidase-conjugated anti-rabbit/-mouse antibodies and a substrate of tetramethyl benzidine. The microplate reader (Bio-Rad, USA) was used to read the optical density at 450 nm. The antibody titers were defined as the greatest degree of sample dilutions which showed 2-fold the absorbance of the control.

IgG was purified using a protein-A chromatography column (Yeasen Biotech company, China) and verified by SDS-PAGE. The concentration of immunoglobulins was determined by Nanodrop™ 2000.

Binding activity of polyclonal antibody with *L. intracellularis*: The binding activity of purified polyclonal antibody with *L. intracellularis* was evaluated by an indirect immunofluorescence assay (IFA). Porcine intestinal epithelial cells (IPEC-J2) were seeded on 6-well plates and infected by *L. intracellularis* with a multiplicity of infection (MOI) 1. *L. intracellularis* infected cell was cultured with DMEM supplement 5% fetal bovine serum and with conditions of 6% O_2, 6% CO_2, and 88% N_2 (Stills, 1991) at 37°C. At 72 h post-infection, the cells were washed with PBS, fixed with 4% paraformaldehyde, and permeabilized with 0.1% TritonX-100 (Sigma, USA). Then cells were incubated with anti-LI0902 and LI1024 polyclonal antibodies overnight at 4°C. After discarding the supernatant and washing, the cells were incubated with the fluorescein isothiocyanate (FITC) conjugated anti-mouse antibodies (Thermo-fisher, USA) and Tetramethylrhodamine (TRITC) conjugated anti-rabbit antibodies (Abcam, USA). The cell nuclei were stained with 4, 6-diamidino-2-phenylindole (DAPI). Then the cells were observed by using a confocal laser scanning microscope at wave-lengths of 358 nm, 488 nm, and 549 nm respectively.

In the parallel assay, the *L. intracellularis* infected cells were scraped from the culture flask and centrifuged with 1000 g for 10 min. The cell pellets were fixed with 3% glutaraldehyde and used to make the ultrathin sections. After staining with sodium phosphotungstate (3%, pH 7.4), the ultrathin sections were observed by transmission electron microscope (TEM).

Preparation of immunomagnetic nanobeads: Magnetic nanobeads (diameter 800 nm, 3 mg/mL) were activated by the 1-ethyl-3-[3-dimethyl aminopropyl] carbodiimide hydrochloride (EDC) and *N*-hydroxy succinimide (NHS) as described previously (Yang *et al*., 2015b). The activated beads were mixed with 200 µg of protein-A with 120 rpm/min of shaking at 37°C for 2 h. After washing with PBS, protein-A coated beads were incubated with 1 mg of anti-LI1024 antibody at 37°C for 1 h. Finally, the immunomagnetic nanobeads (IMNBs) were suspended in PBS containing 1 % BSA (m/v) and 0.05 % NaN_3 (m/v) and stored at 4°C.

Binding activity of IMNBs with *L. intracellularis*: *L. intracellularis* was incubated with IMNBs at 37°C for 1h. Then the IMNBs-bacteria complexes were separated with a magnetic shelf. After 4 times washing with PBST, the IMNBs-bacteria complexes were incubated with an anti-LI1024 antibodies and TRITC-conjugated anti-rabbit antibodies, respectively. After washing, the IMNBs-bacteria complexes were observed directly by confocal microscope at wavelength 549 nm. Meanwhile, the fluorescence intensity was measured by using SpectraMax M5 (molecular devices, USA).

UNPROOF

Rapid detection protocol of *L. intracellularis* by qPCR based on IMNBs: IMNBs were mixed with the samples and incubated at 37°C for 1h. After separation and washing, the IMNBs-bacteria complexes were re-suspended in DNase and RNase-free water, which was used as the template for qPCR testing. The primers *asp*Af: 5`- GCTACACAAGATACGGGTGCT-3`, and *asp*Ar: 5`-TTGAGGAACCAGGTGCCCTTG-3`, target the *asp*A genes were used for qPCR. *asp*A gene was cloned into the pmd18-T vector and used as an amplification standard (Wattanaphansak *et al*., 2010). The cycling parameters were 95°C pre-denaturation for 5 min, 40 cycles of 95°C denaturation for 20 sec, 56°C annealing for 30 sec, and 72°C extension for 20 sec.

Capture efficiency: For detecting the maximal capture dose of IMNBs, approximately 10^6 copies/mL of *L. intracellularis* were incubated with 5 µg of IMNBs at 37°C for 1 h. The IMNBs-bacteria complexes were separated and resuspended in 10 µL of DNase and RNase-free water. The copy numbers of *L. intracellularis* captured by IMNBs were detected by qPCR.

Specificity and detection limit: Several porcine intestinal diseases associated pathogens available in our laboratory, including *Escherichia* (*E.*) *coli* (ATCC25922), *Brachyspira* (*B.*) *hyodysenteriae* (China Institute of Veterinary Drugs Control), *Clostridium* (*C.*) *difficile* (ATCC43593), and porcine epidemic diarrhea virus (PEDV) were used to evaluate the specificity of the qPCR based on IMNBs. Those pathogens were incubated with IMNBs and detected directly by qPCR. The qPCR reaction was confirmed by agarose gel electrophoresis.

L. intracellularis suspension was obtained from the infected IPEC-J2 cultures explained by (Resende *et al*., 2019). 2 µL of the suspension was stained with sodium phosphotungstate and observed by TEM. Serial 10-fold diluted *L. intracellularis* suspension (200 µL) was incubated with 2 µg of IMNBs and subjected to qPCR detection. Meanwhile, bacterial DNA was extracted from the same volume of diluted *L. intracellularis* suspension by DNA extraction kit (Qiagen, Germany) and detected by qPCR.

Clinical samples detection: Faecal samples were collected from different pig farms in China. Twenty samples (PEDV positive confirmed by PCR) were collected from two pig farms of pre-weaning pigs showed symptoms of watery diarrhea. Fifteen samples (Rotavirus positive and PEDV negative confirmed by PCR) were collected from fattening pigs showing signs of diarrhea with mucosanguineous faeces. Fifteen samples were collected from fattening pigs on the same pig farm but breeding in different pig houses without specific symptoms. Sixteen samples were collected from fattening pigs with mild diarrhea (Table 1). The faecal samples were detected by the qPCR based on IMNBs.

Statistical analysis: Student's t-test was used to evaluate the statistical significance of the fluorescence intensity of IMNBs and *L. intracellularis* co-incubation group with the blank IMNBs and PBS control groups.

RESULTS

Expression and purification of recombinant LI0902 and LI1024: The genes of LI0902 and LI1024 were cloned into PET-32a+ vector, expressed in *E. coli*, and analyzed by SDS-PAGE. Our results showed that the expressed and purified recombinant LI0902 and LI1024 have the expected molecular weights of 39 kDa and 60 kDa (Fig. 1A).

Preparation of anti-LI0902 and LI1024 polyclonal antibody: New Zealand White Rabbit and BALB/C mice were immunized as shown in Fig. 1B. Polyclonal titers of post-immunization were verified by an ELISA. Polyclonal titer for both LI0902 and LI1024, immunized animal sera were estimated to be more than $1{:}2^{18}$ (Fig. 1C). The antibodies in serum were purified by protein-A chromatography column. Our results revealed that the purified polyclonal antibodies had the expected molecular weights of the heavy chain of about 55 kDa and the light chain of about 25 kDa (Fig. 1D).

Binding activity of purified polyclonal antibody: *L. intracellularis*-infected IPEC-J2 was detected for the bacteria by an IFA based on purified polyclonal antibodies. The IFA result suggested that *L. intracellularis* was able to be targeted by both anti-LI0902 and anti-LI1024 polyclonal antibodies in IPEC-J2 cells (Fig. 2A, B). To confirm the imaging result based on the polyclonal antibodies, the ultrathin section of *L. intracellularis*-infected IPEC-J2 cells was observed by TEM. *L. intracellularis* organisms, with a length of about 1.25 µm, were evident within the cytoplasm of IPEC-J2 cells that were consistent with the results of IFA (Fig. 2C).

Rapid detection of *L. intracellularis* based on IMNBs: The IMNBs were fabricated and verified by a fluorescence approach. Meanwhile, IMNBs were used in the qPCR approach for *L. intracellularis* rapid detection by the principle shown in Fig. 3A. In the fluorescence methods, the IMNBs captured bacteria can be visualized by confocal laser scanning microscope at wavelength 549 nm based on anti-LI1024 polyclonal antibodies and TRITC-conjugated anti-rabbit antibodies (Fig. 3B). The optical intensity of IMNBs and *L. intracellularis* co-incubation group was higher significantly than the PBS control and blank IMNBs groups (Fig. 3C).

Table 1: Detection of *L. intracellularis* in faecal samples from pig farms

Farm	Location (province)	Clinical signs	Age	Other pathogens	Positive /Total
A	Gansu	Watery diarrhea	Pre-weaning	PEDV +	0/10
B	Shandong	Watery diarrhea	Pre-weaning	PEDV +	0/10
C	Guangdong	Diarrhea with mucosanguineous feces	Fattening	PEDV -, Rotavirus +	5/15
C	Guangdong	No specific clinical sign	Fattening	PEDV -, Rotavirus -	3/15
D	Hebei	Mild diarrhea	Fattening	PEDV -, Rotavirus -	6/16

+, represent positive. -, represent negative.

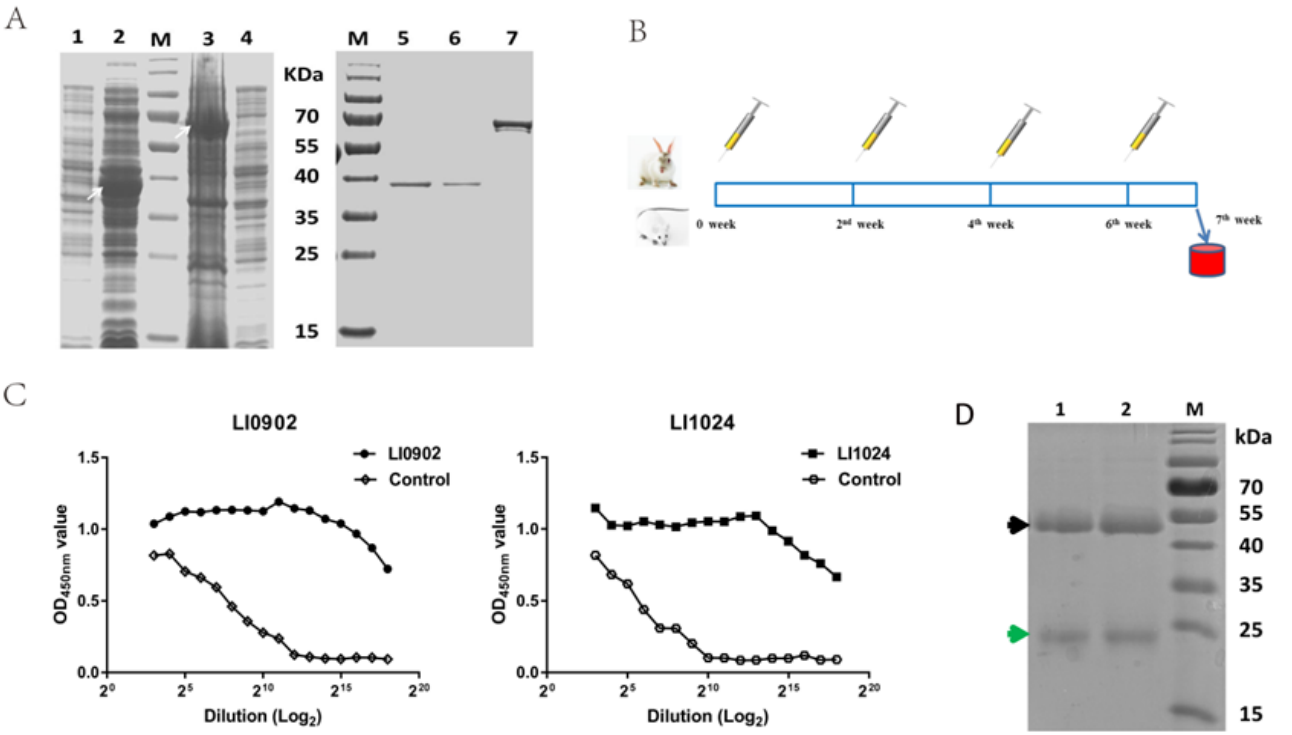

Fig. 1: Expression and polyclonal antibody preparation of membrane protein of *L. intracellularis*; **A:** Expression and purification of membrane protein. Lanes 1 and 4, blank vector control. Lane 2, expression of LI0902. Lane 3, expression of LI1024. Lanes 5 and 6, the purification of LI0902. Lane 7, purification of LI1024. Lane M, protein MW markers. **B:** Schematic representation for immunizing animals with the recombinant LI0902 and LI1024. **C:** ELISA of polyclonal mouse and rabbit serum response to LI0902 and LI1024 respectively. **D:** purification of IgG from immunized animal serum by protein-A affinity resins. Lanes 1, anti-LI0902 polyclonal antibody. Lane 2, anti-LI1024 polyclonal antibody. Heavy chain marked by black arrow, light chain marked by green arrow.

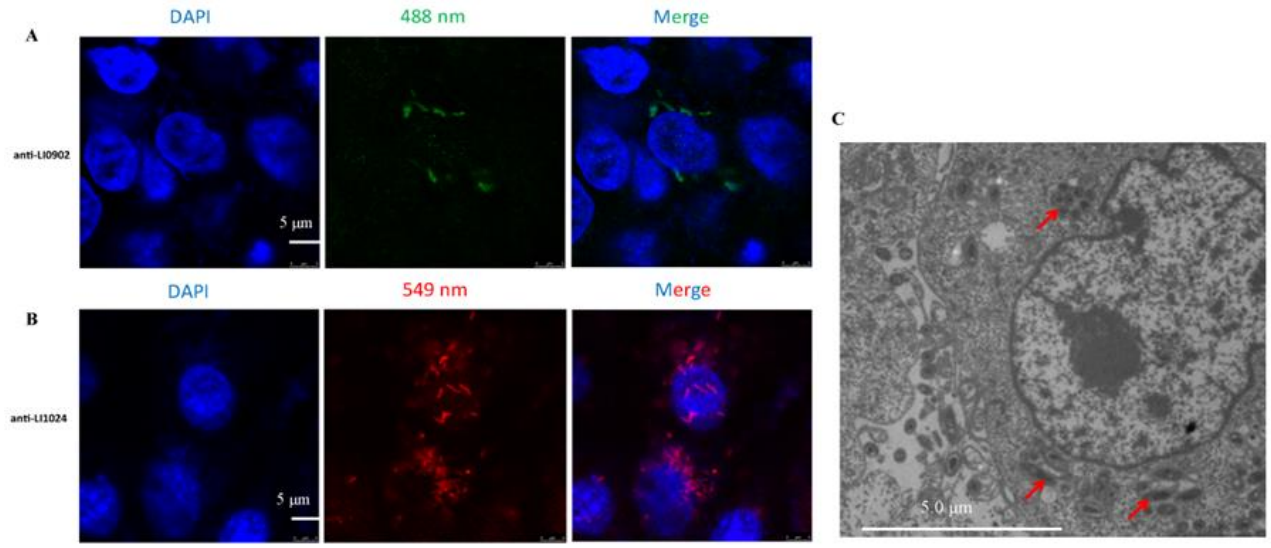

Fig. 2: Immunofluorescence assay for *L. intracellularis* detection base on LI0902 and LI1024 specific antibodies; *L. intracellularis* in porcine intestinal epithelial cells (IPEC-J2) was detected by IFA based on LI0902 and LI1024 specific antibodies. **A:** IFA based on anti-LI0902 polyclonal antibodies and FITC-conjugated anti-mouse antibodies. **B:** IFA based on anti-LI1024 polyclonal antibodies and TRTIC-conjugated anti-rabbit antibodies. The images were viewed in wavelength 488 nm and 548 nm by confocal microscopy. **C:** Ultrathin sections of *L. intracellularis* infected cells was detected by TEM as a parallel control (*L. intracellularis* in cells was marked with red arrows).

Capture efficiency assessment by qPCR: To assess the efficiency of IMNBs, qPCR was conducted on the primer target *asp*A gene. The product of the reaction showed high specificity without primer–dimer formation and the coefficient of determination (R2) was approach to 1 (0.988) when detecting with the standard plasmid (Fig. 4A). To investigate this further, various volumes of *L. intracellularis* were incubated with IMNBs to detect changes in qPCR levels. The capture efficiency of IMNBs was calculated according to the qPCR results. Results showed that 4.21×10^5 copies of *L. intracellularis* were bound by 1 μg of IMNBs (Fig. 4B).

Specificity and detection limit: To analyze the specificity of the qPCR method based on IMNBs, some intestinal disease-associated pathogens, including *E. coli*,

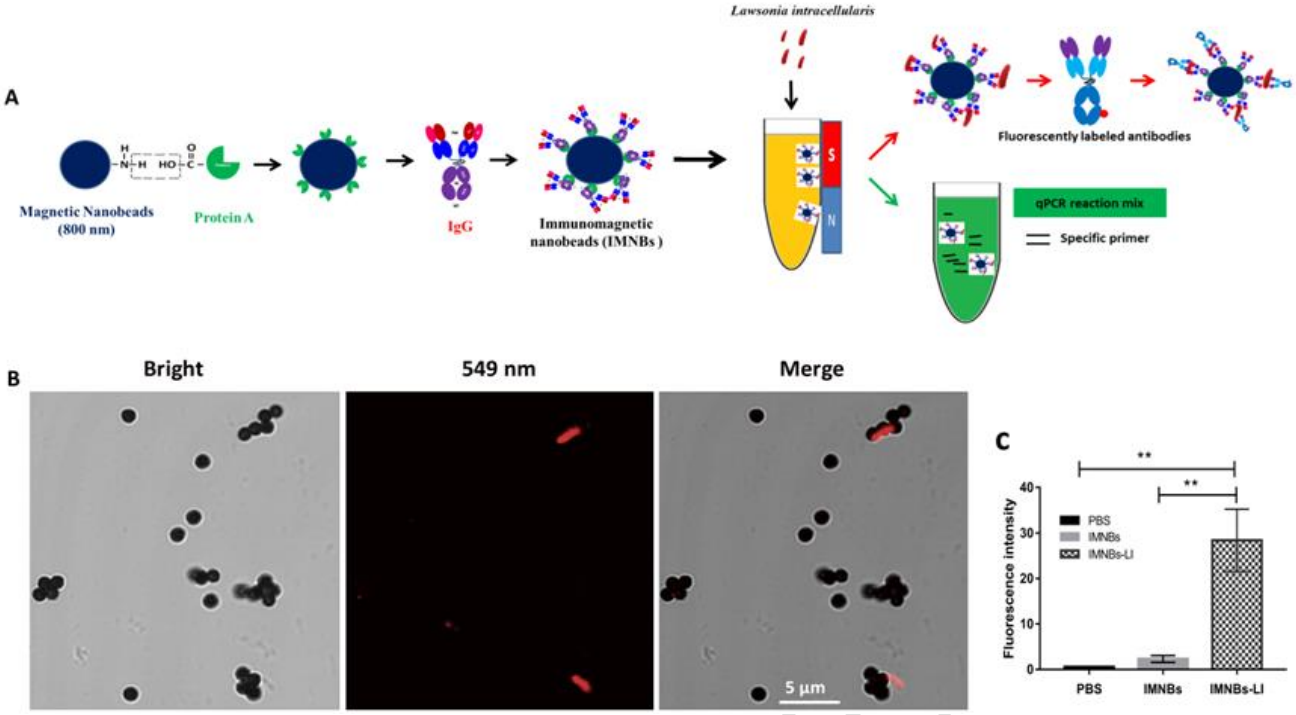

Fig. 3: Visualization of *L. intracellularis* base on IMNBs separation; **A:** Principle of the fabrication and application of IMNBs. IMNBs were fabricated by using the protein A modified magnetic nanobeads and *L. intracellularis* specific antibodies (anti-LI0902 polyclonal antibodies). The captured *L. intracellularis* can be detected based on fluorescently labeled antibodies or qPCR directly. **B:** The captured *L. intracellularis* was visualized by using the anti-LI1024 antibodies and TRTIC-labeled anti-rabbit antibodies. The images were viewed in bright field and wavelength 549 nm by confocal microscopy. **C:** The fluorescence intensity was measured by SpectraMax M5. The data are expressed as the mean ± SEM of three times repeats.

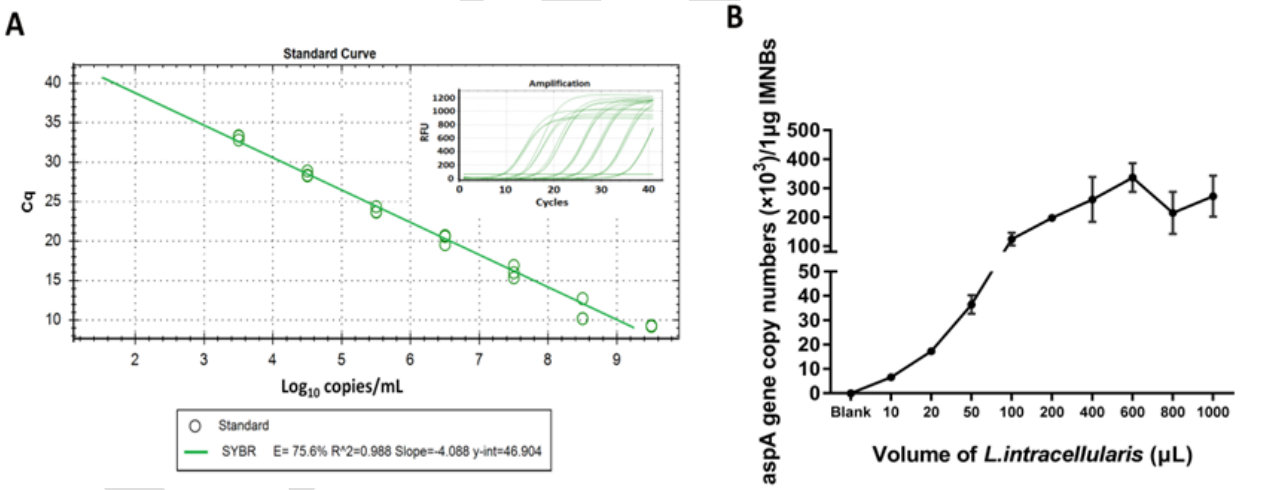

Fig. 4: Capture efficiency of IMNBs to *L. intracellularis*; **A**: Calibration curve used for *asp*A gene copies calculating in qPCR. **Insert:** amplification curve. The data are expressed as the mean ± SEM of three times repeats. **B**: 4 μg of IMNBs was incubated with different volume of *L. intracellularis*, then the *asp*A gene copies of *L. intracellularis* in detached IMNBs-bacteria composites was determined by a qPCR.

B. hyodysenteriae, *C. difficile*, and PEDV were used as controls. Approximate 3×10^5 copies of *asp*A gene with an amplification size of 160bp (Fig. 5) were showed in the *L. intracellularis*-positive sample. In contrast, no gene copies and amplification were found in the other pathogen samples (Fig. 5).

To determine the detection limit, serial dilutions of *L. intracellularis* obtained from *L. intracellularis*-infected IPEC-J2 cells, which were verified by TEM, were tested using this method. These organisms are straight or slightly curved cylindrical rods with a length of around 1.25 μm and a diameter of approximately 0.3 μm and were easily observable in the suspension (Fig. 6A). The qPCR assay base on IMNBs provided a limit of detection as low as 3.6 $\times 10^2$ copies/mL that was similar with conventional qPCR of 2.78 $\times 10^2$ copies/mL (Fig. 6B).

Clinical samples detection: Faecal samples of (n=66) pigs from three pig farms with clinical signs of watery diarrhea, diarrhea with mucosanguineous feces, mild diarrhea, or no symptoms were detected using this method. Faecal samples (n=20) with watery diarrhea were PEDV positive and *L. intracellularis* negative. Five out of fifteen (5/15) mucosanguineous faecal samples confirmed rotavirus positive, three out of fifteen (3/15) no clinical sign samples, and six out of sixteen mild diarrhea faecal

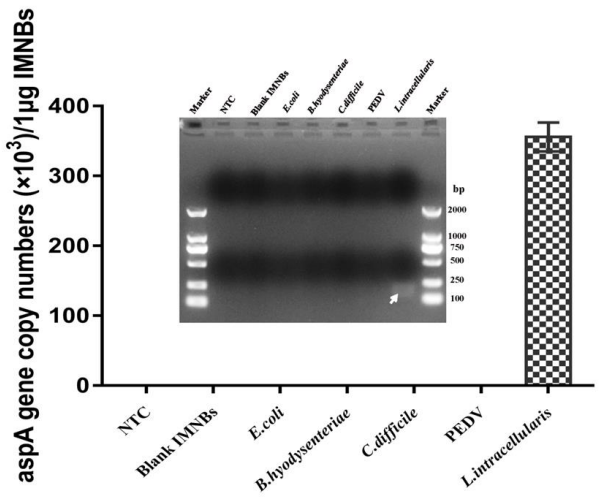

Fig. 5: Specificity of the qPCR based on IMNBs; Porcine intestinal diseases associated pathogens *E. coli*, *B. hyodysenteriae*, *C. difficile*, and PEDV were used for the assessment of the specificity of the qPCR based on IMNBs. **Insert:** The image of agarose gel electrophoresis of the qPCR reactions. The data are expressed as the mean ± SEM of three times repeats.

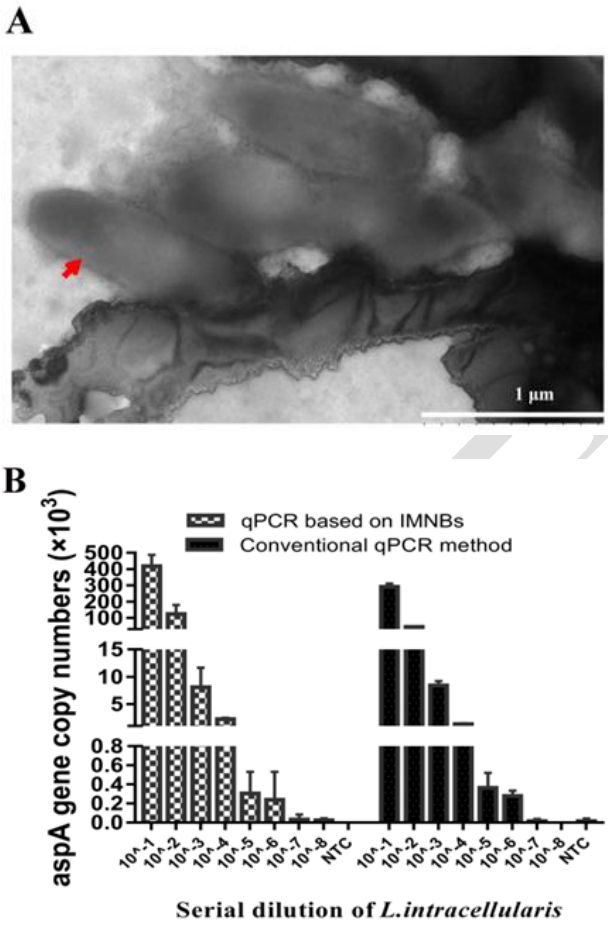

Fig. 6: Detection limits of the qPCR based on IMNBs; **A:** *L. intracellularis* suspension was verified by TEM. *L. intracellularis* organisms were marked with red arrows. **B:** 200 µL of serial 10-fold diluted *L. intracellularis* was incubated with IMNBs and detected by qPCR. Meanwhile another 200 µL of serial 10-fold diluted *L. intracellularis* was used for bacterial DNA extraction and detected by qPCR. The data are expressed as the mean ± SEM of three times repeats.

samples were *L. intracellularis* positive (Table 1). The results indicate that the method qPCR combined with IMNBs is suitable for rapid detection of *L. intracellularis* in the clinical faecal sample.

DISCUSSION

L. intracellularis is an enteric pathogen to various animal species, including mammals and birds (Lawson and Gebhart, 2000). *L. intracellularis* infection results in diarrhea and intestinal lesions, which have a huge impact on pig growth and feed conversion. Therefore, PPE caused by *L. intracellularis* is now acknowledged as one of the most economically important diseases in the global swine industry. Rapid and reliable *L. intracellularis* detection on pig farms helps prevent and control PPE by guiding vaccinations and emergency treatment. This study designed and assessed a reliable *L. intracellularis* detection method using IMNBs and qPCR.

Immunomagnetic beads have been widely used to capture and separate viruses, bacteria, parasites, and tumor cells (Hu *et al*., 2021; Khosravi *et al*., 2021; Tayachi *et al*., 2021). The capture activity of immunomagnetic beads depends on the binder such as the antibodies which are conjugated on its surface. To achieve target-specific separation, the selected antibodies must exhibit excellent affinity and specificity to the target antigen. Polyclonal antibodies have been extensively used in etiological detection methods, including enzyme-linked immune\sorbent assay, immunoblotting, and agar immunodiffusion reaction, due to their exceptional affinity for the target antigen. In this investigation, LI0902 and LI1024 proteins, the outer membrane protein of *L. intracellularis* (Watson *et al*., 2014), were recombinant expressed and employed to generate polyclonal antibodies in rabbits and mice. The serum polyclonal antibodies were purified and conjugated with magnetic nanobeads following the determination of the *L. intracellularis* binding activity. Antibodies conjugated oriented on the surface of magnetic microspheres can significantly improve capture activity (Anderson *et al*., 2019). Thus, in this study, the magnetic nanobeads were conjugated with protein-A which could keep antibodies oriented binding on the surface of IMNBs. The IFA and qPCR tests suggest that IMNBs have *L. intracellularis* capture activity with a high efficiency of 4.21×10^5 copies of *L. intracellularis* per 1 µg of IMNBs.

The subsequent phase of the procedure involves exhibiting the acquired microorganisms using immunomagnetic nanoparticles (IMNBs). PCR is a commonly used technology for identifying the cause of a disease, known as etiology. It is known for its ability to accurately detect the exact cause of a disease with high levels of sensitivity and specificity. Recent research has shown that PCR testing is effective in identifying *L. intracellularis* in the clinical faecal samples (Jacobson *et al*., 2009; Nathues *et al*., 2009; Pedersen *et al*., 2010). So, a qPCR target *asp*A gene, one copy located on the chromosome of *L. intracellularis* (Wattanaphansak *et al*., 2010), was developed and optimized in this study. The primer used for the qPCR of this work had a particular product without dimer and had a high qPCR efficiency approach to 100%, with an R^2 value approach to 1 in the standard curve based on the standard plasmid.

Conventionally, the first step of traditional PCR determination techniques for clinical samples is the extraction of the pathogen's nucleic acid (DNA/RNA). This process can be lengthy and there is a potential risk of nucleic acid contamination. Taking these for consideration, in this study the IMNBs captured by *L. intracellularis* were used as a model directly for the qPCR test. Compared with conventional PCR methods, IMNBs reduce operating steps, instruments, and processing times, since only a magnetic scaffold and several minutes are required. To check the specificity and sensitivity of this technique, we tested several intestinal pathogens from pigs in our lab, such as *E. coli*, *B. hyodysenteriae*, *C. difficile*, and PEDV. The results indicate that this method had good specificity and a high sensitivity of 3.6×10^2 copies per 1 mL, comparable to conventional qPCR (Wattanaphansak *et al*., 2010).

The detection of *L. intracellularis* in faeces was a common way for the routine diagnosis of PPE and the epidemiological investigation (Baldasso *et al*., 2023; Luppi *et al*., 2023). In this study, faecal samples (n=66) from pigs with different clinical signs were detected by using the method of qPCR combined with IMNBs. The results showed that n=14 samples were *L. intracellularis* positive, including 5 from TGEV-infected pigs, 6 from mild diarrhea pigs, and 3 from healthy pigs. The results indicated that the method of qPCR combined with IMNBs is suitable for the rapid detection of *L. intracellularis* in clinical faecal samples. This study provides a robust method for rapid diagnosis and epidemiological investigation of *L. intracellularis.*

Conclusion: In this study, the outer membrane protein of *L. intracellularis* was recombinantly expressed and used to make polyclonal antibodies. The IMNBs with *L. intracellularis* capture activity were assembled successfully using outer membrane protein-specific polyclonal antibody conjugated with magnetic nanobeads. Then, a sensitive and rapid method for *L. intracellularis* detection was developed by using qPCR combined with IMNBs, which showed good specificity and sensitivity. Furthermore, using IMNBs combined with qPCR greatly simplified the manipulation of *L. intracellularis* detection. Moreover, the results of the clinical samples detection have validated the promising prospect of this method for efficient detection of *L. intracellularis* in clinical faecal samples.

Ethics statement: This study was approved by The Animal Administration and Ethics Committee of the College of Animal Science and Technology, Hebei Normal University of Science and Technology. Mice and rabbits were bred in animal houses. Immunization and serum collection were strictly under good animal practice according to the Animal Ethics Procedures and Guidelines of the People`s Republic of China. This study did not raise any ethical issues.

Competing interests: The authors declare no competing interests.

Authors' contributions: Yang, SL participated in planning the study, conducted most of the experiments, and drafted the manuscript. Khan MUZ helped in the ELISA and TEM. Pan, SM and Gai WY helped in the collection of clinical samples. Zhang XQ helped in test of clinical samples. Zhang J conceived of the study and helped draft the manuscript. Liu, YS gave some scientific advice and revised the manuscript. All authors read and approved the final manuscript.

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