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Development of Rapid Detection Kit for Necrotic Enteritis Disease in Poultry using Protein A Agglutination

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ABSTRACT

Necrotic enteritis causes significant losses in the global poultry industry, necessitating accurate diagnosis for effective intervention. This study aimed to develop a diagnostic tool for detecting necrotic enteritis in poultry based on the presence of *Clostridium perfringens* (*C. perfringens)* Alpha-toxin in poultry feces. The reagent of the detection kit was developed by conjugation of IgG against *C. perfringens* toxin and *Staphylococcus* cells containing protein A. The IgG antibody was derived from an 8-month-old thin-tailed male sheep immunized with purified 2 ml of *C. perfringens* Alpha-toxin. Sensitivity assays were carried out to determine the detection limit, while *Escherichia coli* (*E. coli*) and *Salmonella enteritidis* (*S. enteritidis*) were used to identify specificity. A purified Alpha-toxin with a protein concentration of 2.8 mg/ml and a specific molecular weight of 43 kDa was successfully obtained. A strong reaction of the hyperimmune antibody (IgG) was also detected in the thin-tailed male sheep serum. The developed rapid detection kit in this study indicated *C. perfringens* Alpha-toxin with a lower concentration (12 ng/ml). Agglutination reactions could differentiate positive control from negative without significant cross-reactivity towards other bacteria (*S. enteritidis* and *E. coli*).

Keywords: Agglutination, *Clostridium perfringens*, Detection, Necrotic Enteritis, Toxin

INTRODUCTION

Necrotic enteritis, among enteric diseases, inflicts substantial losses by reducing production and increasing mortality, leading to an annual financial setback of around $6 billion for the global poultry industry (Wade and Keyburn, 2015). This poultry affliction is characterized by lesions scattered throughout the small intestine, contributing to growth retardation, reduced feed efficiency, and a mortality rate ranging from 10% to 40% within a single flock (Lacey et al., 2018; Zahoor et al., 2018). The subclinical manifestation of the disease results in decreased body weight or impaired body weight gain and adversely affects the feed conversion ratio. Necrotic enteritis is a complex ailment, with coccidia infection standing out as a crucial predisposing factor due to its detrimental impact on intestinal epithelial integrity, thereby promoting the colonization of *C. perfringens* (Shojadoost et al., 2012; Moore, 2016).

Over the years, antibiotic growth promoters (AGPs) and anticoccidial drugs have been widely used to uphold intestinal health and mitigate disease risks, especially in the context of intensive commercial production conditions (Daniel et al., 2011). Nevertheless, the extensive application of antimicrobials in animal feed is now considered imprudent due to concerns about its potential contribution to antibiotic-resistant pathogenic bacteria that could be transmitted to humans (Seal et al., 2013; Broderick et al., 2021). Consequently, it is pivotal to diagnose necrotic enteritis through observations in both field and laboratory animal studies. This diagnosis is crucial for determining disease control and intervention strategies, such as vaccination (Abd El-Hack et al., 2022).

Diagnosis of necrotic enteric diseases poses challenges. A preliminary assessment can be made by considering the flock's history and gross lesions. Necrotic enteritis diagnosis was confirmed by histopathology due to the damage of intestinal epithelium and lesion in the duodenum, jejunum, ileum, and cecum. Acute lesions include friability of the

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small intestine, filled with reddish or dark brown pseudo membranes over the mucosal-associated with multifocal to coalescent ulcers (Santiani et al., 2023). However, this method requires a series of processes, specific tools, specialized laboratories, and professional interpretation.

Accurate identification of clostridial enteric disease is challenging, particularly for lacking familiarity with this specific disease or other common poultry enteric diseases such as coccidiosis. Different interpretations exist regarding the implications and visual characteristics of intestine autolytic changes, particularly at the microscopic level. These changes start shortly after death and are often mistaken for pathological alterations (Smyth, 2016). In the current study, a diagnostic tool kit for necrotic enteritis was developed to detect the presence of *C. perfringens* Alpha-toxin in the feces of poultry suspected of being infected with the disease. Sensitivity and specificity tests were also carried out to determine the potential, benefits, and effectiveness of the kit in the field.

MATERIALS AND METHODS

Ethical approval

All research methods and practices and the use of animals have been approved by the Animal Ethics Committee, School of Veterinary Medicine and Biomedical Sciences, Institut Pertanian Bogor (IPB) University, Bogor, Indonesia, with certificate number 096/KEH/SKE/VIII/2023.

Study time, location, and animal

This research was conducted at various locations from January to August 2023. The production and purification of alpha toxin from *C. perfringens* were carried out at the research and development (RND) unit of PT. Medika Satwa Laboratoris, Indonesia. Antibody production was performed using 8-month-old thin-tailed male sheep in the animal testing facility of PT. Medika Satwa Laboratoris, Indonesia. The manufacturing and testing of the detection kit were conducted at the Laboratory for Development and Production of Biological Materials at IPB University, Indonesia.

Clostridium perfringens alpha-toxin production and purification

The production of alpha-toxins from *C. perfringens* was conducted in accordance with the previous study (Kurnia et al., 2022) to obtain crude toxin. Subsequently, the potential toxin activity was determined based on hemolytic activity with readings on spectrophotometry (UV-Vis Spectrophotometry Genesys, Thermo Scientific, USA) at a wavelength of 540 nm. Furthermore, the crude toxin was purified through several stages, starting with ammonium sulfate precipitation to obtain a protein toxin concentrate. This was followed by ion exchange, where the toxin protein fractions were determined based on hemolytic activity, concentration, and molecular weight (Ochi et al., 2004; Duong-Ly and Gabelli, 2014).

Antibodies production

The purified toxin protein was injected subcutaneously into 8-month-old thin-tailed male sheep twice, at a 28-day interval, to obtain serum containing IgG of anti-alpha-toxin. Antibodies were collected from sheep that had previously hyperimmunized. Five native sheep with an average weight of 55 ± 1.5 were injected with 2 ml of toxoid. After 2 weeks of the second injection, the immunity level of each animal was determined from each serum by antigen-antibody reaction using an agar gel immunodiffusion assay. The purification of IgG was performed using the addition of rivanol (2-ethyoxy-6,9-diaminoacridine lactate) followed by ammonium sulphate precipitation (Vargas et al., 2012). Subsequently, the concentration and purity of IgG from serum were measured using UV-Vis Spectrophotometry Genesys, Thermo Scientific, USA at 260/280 nm wavelength.

Preparation and IgG conjugation of Protein A

In the current study, *Staphylococcus aureus* (*S. aureus*) strain Cowan I (ATCC 12598) obtained from IPB University was used to produce large amounts of protein A. The preparation of stabilized staphylococci followed a previously established protocol that employed protein A from *S. aureus* cells to facilitate binding IgG, ensuring that the antigen-antibody reaction manifested as co-agglutination (Arnafia et al., 2017). *S. aureus* Cowan I was grown in blood agar at 37°C for 24 hours. The bacterial cells were collected by centrifugation (10000× g, 20 minutes), followed by three washes with phosphate buffer saline (PBS, pH 7.4). The cells were then suspended in 0.5% formalin-PBS (v/v) solution and incubated for 3 hours at 25°C. Subsequently, the cells were washed with PBS and resuspended in PBS at a concentration of 10% (v/v). The suspension was then heated at 80°C for an hour, washed three times with PBS, and resuspended in PBS at the concentration of 10% (v/v). The cells were stained by resuspending in 10% crystal violet. After incubation for 20 minutes at 25°C, the cells were filtered in sterile cotton, centrifuged at 10.000× g for 20 minutes,

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and resuspended in PBS-sodium azide 0.1% at the concentration of 10% (v/v). Monospecific IgG of anti-alpha-toxin with a concentration of 2.1 mg/ml were mixed with 300 µl stabilized *Staphylococcus* cells and allowed to attach for 3 hours at 37℃. The suspension was centrifuged at 5000× g for 5 minutes and washed twice with 0.02 M phosphate (pH 7.3)-buffered 0.85% saline (PBS). The cells were then suspended in PBS to achieve a final volume of 1 ml. The antibody-conjugated staphylococci were then stored at 4°C until they were used as direct co-agglutination reagents.

Sensitivity and specificity test

In the current study, the sensitivity of a kit prototype to detect alpha-toxin of *C. perfringens* was evaluated using an agglutination reaction (Kurnia et al., 2022). The purified *C. perfringens* alpha toxin was diluted in PBS so that graded concentrations of the toxin could be tested to determine sensitivity (detection limit) in suspension. In the experiment, 15 µL from each concentration of toxin suspension was mixed with 15 µL of kit reagent on a glass slide. The agglutination reaction was examined for 30 seconds. *Salmonella enteritidis* (*S. enteritidis*) ATCC 13076, and *Escherichia coli* (*E. coli*) ATCC 25922 bacteria isolates were used to determine the specificity (cross-reactivity) of the kit prototype. All tested bacteria were cultured on a blood agar medium for 24 hours at 37°C. A single colony from each tested bacteria was suspended in 10 µL of PBS, mixed with 10 µL kit reagent on a glass slide, and observed for 10 seconds. Specific gene detection by PCR was compared with the result of agglutination to determine the specificity of the kit prototype. Fresh overnight bacterial cultures in the brain heart infusion (BHI) were used for nucleic acid extraction using the boiling method. All the DNA extracted from the bacteria was examined using PCR with a specific primer. The primer sequence of specific uspA gene *E. coli* was 5′-CCGATACGCCTGCCAATCAGT-3′ and 5′-ACGCAGACCGTAGGCCAGAT-3′ (Rubio et al., 2019). The primer sequence of *S. enteritidis*-specific gene was 5′-ATATCGTCGTTGCTGCTTCC-3′ and 5′- CATTGTTCCACCGTCACTTTG-3′ (Hardiati et al., 2021).

Limit of detection of the development kit in fecal sample

Simulated fecal samples were prepared by adding the *C. perfringens* alpha toxin serial dilutions to negative control poultry feces. The toxin was added to the fecal sample with various concentrations (12000 ng/ml, 1200 ng/ml, 120 ng/ml, 12 ng/ml). Subsequently, 250 µl of the fecal sample was transferred into a microtube and added with 250 µl of 0.2 M AMP (2-Amino-2-methyl-1-propanol) buffer. Then, 500 µl of suspension was centrifuged at 10000× g for 5 minutes. Using a kit prototype, the supernatant was used as a sample for the co-agglutination procedure.

RESULTS

Purification of *Clostridium perfringens* toxins

The findings indicated that ion exchange was effective in purifying the toxin, with the elution step using 0.2 Mol NaCl releasing pure alpha toxin protein fraction based on a higher specific activity of 5.67 U/mg with a protein concentration of 2.8 mg/ml (Figure 1). The specific molecular weight of that protein fraction is also known to be 43 kDa based on observations using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

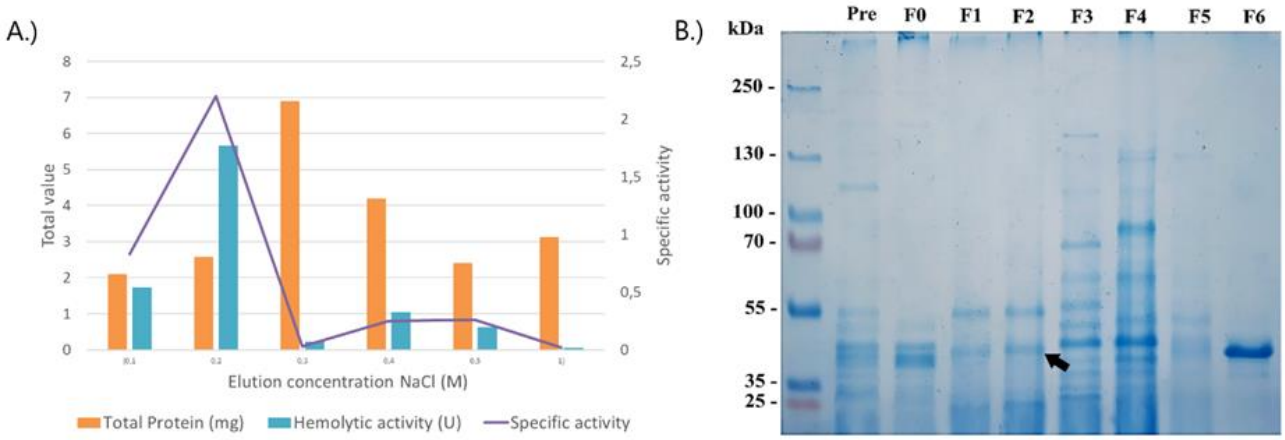

Figure 1. The protein fraction of *Clostridium perfringens* alpha-toxin. **A:** Total value (mg/U) analysis of protein fraction by ion exchange purification at gradual NaCl elution. A higher specific activity value of alpha toxin appears at 0.2 M NaCl concentration. **B:** SDS-PAGE analysis shows that protein fraction 2 (F2) contains specific molecular weight at 43 kDa and 56 kDa. Pre: Crude protein before purification; F0: Dilution of 0 M NaCl; F1: Dilution of 0.1 M NaCl; F2: Dilution of 0.2 M NaCl; F3: Dilution of 0.4 M NaCl; F4: Dilution of 0.6 M NaCl; F5: Dilution of 0.8 M NaCl; F6: Dilution of 1 M NaCl

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Production of antibodies anti-*Clostridium perfringens* alpha-toxin

The antibody obtained from sheep's serum immunized by *C. perfringens* toxin showed a response by an appearance of thin precipitin lines in the second week post-induction (Ab1) at Agar Gel Immunodiffusion Assay. Meanwhile, the precipitin lines appear more distinct in the fourth week and second week post-booster (Ab2 and Ab3). The purification of IgG from the serum indicated a thick single precipitin line (Figure 2). This finding revealed a strong reaction between the toxin antigen and the IgG antibodies obtained.

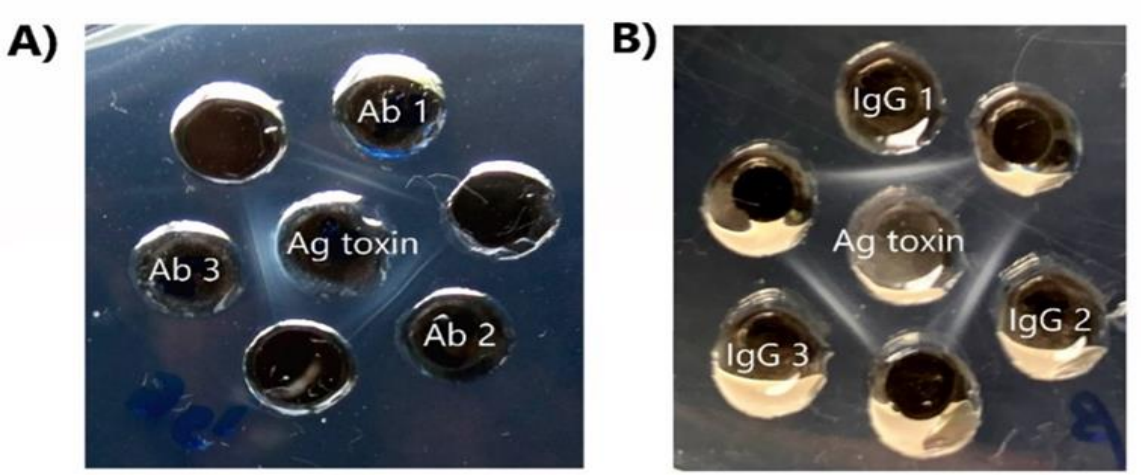

Figure 2. Agar gel immunodiffusion assay reaction of *C. perfringens* alpha toxin and specific antibody. **A:** Antigen and serum in the second-week post-induction (Ab1), reaction in the fourth-week post-induction (Ab2), and second-week post-booster (Ab3). **B:** Antigen and purified IgG in the second-week post-induction (IgG1), the reaction in the fourth-week post-induction (IgG2), and second-week post-booster (IgG3).

Sensitivity and specificity of agglutination kit reagent

Conjugation between IgG antibodies against *C. perfringens* toxin and *Staphylococcus* cell wall was observed through an agglutination reaction when the *C. perfringens* Alpha-toxin was added to the reagent as a control. To assess sensitivity, decreasing concentrations were tested. Consequently, the agglutination reaction became increasingly faint as the concentration of the control toxin decreased. At this stage, it can be determined that the agglutination reaction can detect the presence of the toxin with a lower concentration based on serial dilutions of the *C. perfringens* alpha toxin at approximately 12 ng/ml (Figure 3A).

Table 1. The comparison of co-agglutination reaction results for the detection of *Clostridium perfringens* alpha-toxin with PCR results to confirm the bacterial identity

Bacterial antigen	Agglutination reaction	PCR with specific primer
Clostridium perfringens alpha-toxin	+	-
Salmonella enteritidis ATCC	-	+
Escherichia coli ATCC	-	+

ATCC: American Type Culture Collection

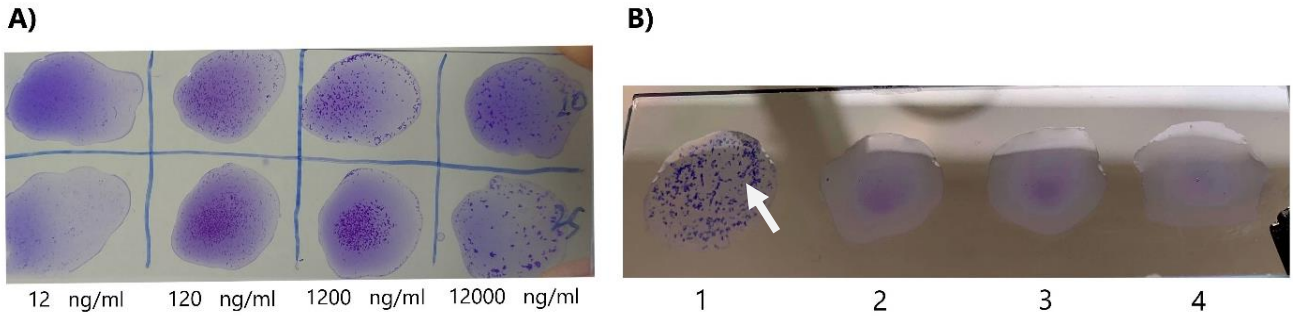

Figure 3. Agglutination reaction of the developed reagent. **A:** Sensitivity of the reagent by decreased toxin (12 µg/ml – 12 ng/ml) shows that the agglutination reaction becomes increasingly faint. **B:** Determination of specificity (cross-reactivity) of kit prototype, agglutination reaction of *Clostridium perfringens* alpha with reagent (1); reaction of *Salmonella enteritidis* with reagent (2); reaction of *Escherichia coli* with reagent (3); negative control PBS (4).

Determination of agglutination kit reagent specificity was conducted based on a comparison with another

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diagnostic test using PCR (Figure 4). It appeared that no agglutination reaction occurred by adding *E. coli* and *Salmonella* antigens with the reagent, indicating that there is no cross-reactivity with other antigen bacteria (Figure 3B). Negative control by PBS was also carried out to make sure no self-agglutination formation particles occurred in the reaction.

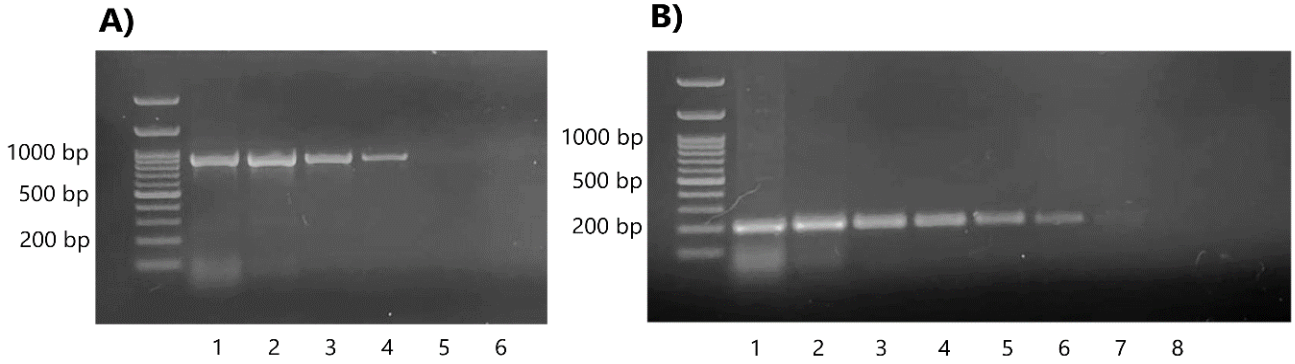

Figure 4. PCR confirmation identity of *Escherichia coli* and *Salmonella enteritidis* by specific primer. **A:** An approximate 880 bp band size represents uspA gene that specifically identifies *Escherichia coli* isolate. Lane 6 is non-template control (NTC), while 1-5 was bacterial genomic DNA template at amounts from 210 ng to 21 pg. **B:** An approximate 206 bp band size represents specific gene of *Salmonella enteritidis*. Lane 8 is NTC, while 1-7 was bacterial genomic DNA template from 193 ng to 19 pg.

DISCUSSION

The rapid detection kit developed in this study utilized a co-agglutination reaction with two primary elements. These components included *S. aureus* cells with protein A (*S. aureus* Cowan I) that act as a foundational matrix, while IgG of anti-alpha-toxin of *C. perfringens* type A serves as the agent to detect antigens within the sample. Protein A of *S. aureus is* known to have a high affinity for the Fc region of IgG (O'Seaghdha et al., 2006). The protein is abundantly present on the bacterial cell wall and composed of a signal sequence. Each of its repeating Ig-binding domains adopts a three-helical structure that can bind to the Fc region of IgG via helices I and II (Cruz et al., 2021). While the Fc region attaches to the protein A structure of *Staphylococcus aureus*, Fab region of IgG detects explicitly the antigen in the fecal sample. The co-agglutination reaction co-occurs with *C. perfringens* type A toxin, resulting from the binding between antigens and antibodies already bound to *Staphylococcus* cell wall.

Digestive tract infections represent a significant concern within the poultry industry and have resulted in substantial economic losses (Salem and Attia, 2021). Necrotic enteritis (NE), whether in clinical or subclinical form, is a major gastrointestinal ailment in poultry, severely impacting profitability in the poultry sector (Bansal et al., 2021; Salem et al., 2021). Necrotic enteritis continues to pose challenges, particularly in regions with poorly managed poultry operations. In the diagnostic process for NE, errors often occur, particularly in the context of bacterial infections. This leads to an upsurge in the use of antibiotics on farms as a response to NE (Fathima et al., 2022). To counteract the increasing consumption of antibiotics, which could potentially contribute to antibiotic resistance, a diagnostic kit for NE has been developed. Recently, various methods, such as ELISA, have been explored and created to rapidly detect NE. However, ELISA comes with certain limitations, such as the need for expensive, highly specialized laboratory equipment and the requirement for well-trained personnel (Nnachi et al., 2022).

The co-agglutination technique has evolved to detect certain bacteria, including toxins from *C. perfringens*. The co-agglutination technique offers a range of advantages, including sensitivity, specificity, speed, simplicity, cost-effectiveness, and reliability (Dong et al., 2019). The construction of a reagent can be optimized to distinguish between positive and negative controls clearly. Sensitivity observation revealed that alpha-toxin from *C. perfringens* can be detected by reagent developed at a lower concentration of 12 ng/ml in the current study. Based on a previous study, the concentration of *C. perfringens* toxin ranged from 0.19 to 17.6 ng per gram of wet feces was found in poultry with confirmed NE lesions, so native toxin was detected in the digestive or fecal droppings (Lee et al., 2020; 2021). This finding supports the idea that the reagent developed in this research will be able to detect *C. perfringens* toxin within the appropriate range concentrations in the field.

Reagents developed in the current study were also assessed for their reaction with other bacteria related to digestive disease. These bacteria, including *E. coli* and *S. enteritidis*, had no cross-reaction based on agglutination observation. A single colony used for current detection indicated the absence of an agglutination. Bacterial identification was further validated by PCR to detect specific genes that identify certain bacteria based on DNA material genetics in gradually

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decreasing concentrations. The diagnosis of diseases caused by bacteria or viruses, with the analysis at the molecular level, poses unique challenges when detecting protein structures like toxins (Pawaiya et al., 2020). In line with the findings of the current study, Serroni et al. (2022) have found that the kit development has the potential to detect toxins in digestive diseases with a rapid reaction, considering toxin protein structures were time-dependent proteolytic degradation itself in fecal samples.

CONCLUSION

The results of the study indicated that the kit developed in this study could detect a reagent for Necrotic enteritis. The conjugation of IgG of anti-alpha toxin of *C. perfringens* type A, with *Staphylococcus* protein A produced an agglutination reaction when samples containing *C. perfringens* toxin were added. The sensitivity of the kit also indicated that the toxin concentration could be detected within the appropriate range in a field case of Necrotic Enteritis, with well-established specificity. However, further studies regarding the stability, with several quality control observations of this diagnostic kit, need to be conducted to determine optimal results.

DECLARATIONS

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Authors' contributions

Ryan Septa Kurnia, Christian Marco Hadi Nugroho, Otto Sahat Marua Silaen, Muhammad Ade Putra, Vivin Aulia Rahmi, Alya Amaliah, Safika, and Agustin Indrawati conceived, designed, collected, and analyzed data and wrote the manuscript. Rani Wardani Hakim, Maxs U.E Sanam, Amin Soebandrio, Safika, Agustin Indrawati designed, supervised the study, and reviewed the manuscript. All authors read and approved the final draft of the manuscript for publication.

Competing interests

The authors declared that there are no competing interests.

Ethical considerations

The authors declare that this manuscript is original and is not being considered elsewhere for publication. Other ethical issues, including consent to publish, misconduct, fabrication of data, and redundancy, have been checked by the authors.

Availability of data and materials

All data of the current study are available upon reasonable requests from the authors.

REFERENCES

Abd El-Hack ME, El-Saadony MT, Elbestawy AR, El-Shall NA, Saad AM, Salem HM, El-Tahan AM, Khafaga AF, Taha AE, AbuQamar SF et al. (2022). Necrotic enteritis in broiler chickens: Disease characteristics and prevention using organic antibiotic alternatives- A comprehensive review. Poultry Science, 101(2): 101590. DOI: https://www.doi.org/10.1016/j.psj.2021.101590

Arnafia W, Ningrum S, Adji RS, Lukman DW, Pasaribu FH, and Wibawan IT (2017). Development of a rapid diagnostic kit for detection of *Salmonella Enteritidis* in food using indirect coagglutination technique. Veterinaria, 66(3): 119-125. Available at: https://veterinaria.unsa.ba/journal/index.php/vfs/article/view/261

Bansal M, Alenezi T, Fu Y, Almansour A, Wang H, Gupta A, Liyanage R, Graham DB, Hargis BM, and Sun X (2021). Specific secondary bile acids control chicken necrotic enteritis. Pathogens, 10(8): 1041. DOI: https://www.doi.org/10.3390/pathogens10081041

Broderick TJ, Gutierrez O, Lee JT, and Duong T (2021). Evaluation of functional feed additive administration in broiler chickens to 21d. Journal of Applied Poultry Research, 30(2): 100121. DOI: https://www.doi.org/10.1016/j.japr.2020.100121

Cruz AR, Boer MAD, Strasser J, Zwarthoff SA, Beurskens FJ, de Haas CJC, Aerts PC, Wang G, de Jong RN, Bagnoli F et al. (2021). Staphylococcal protein A inhibits complement activation by interfering with IgG hexamer formation. Proceedings of the National Academy of Sciences of the United States of America, 118(7): e2016772118. DOI: https://www.doi.org/10.1073/pnas.2016772118

Daniel CR, Cross AJ, Koebnick C, and Sinha R (2011). Trends in meat consumption in the USA. Public Health Nutrition, 14(4): 575-583. DOI: https://www.doi.org/10.1017/S1368980010002077

Dong X, Tao J, Li T, Zhang P, Zhu Y, Tang Y, Su R, and Shi D (2019). A rapid, simple, and sensitive immunoagglutination assay with silica nanoparticles for serotype identification of *Pseudomonas aeruginosa*. Journal of Integrative Agriculture, 18(8): 1936-1943. DOI:

To cite this paper: Kurnia RS, Nugroho CMH, Silaen OSM, Putra MA, Rahmi VA, Amaliah A, Hakim RW, Sanam MUE, Soebandrio A, Safika, and Indrawati A (2024). Development of Rapid Detection Kit for Necrotic Enteritis Disease in Poultry using Protein A Agglutination. *World Vet. J.*, 14 (1): 8-14. DOI: https://dx.doi.org/10.54203/scil.2023.wvj2

https://www.doi.org/10.1016/S2095-3119(18)62135-5
Duong-Ly KC and Gabelli SB (2014). Using ion exchange chromatography to purify a recombinantly expressed protein. Methods in Enzymology, 541: 95-103. DOI: https://www.doi.org/10.1016/b978-0-12-420119-4.00008-2
Fathima S, Hakeem WGA, Shanmugasundaram R, and Selvaraj RK (2022). Necrotic enteritis in broiler chickens: A review on the pathogen, pathogenesis, and prevention. Microorganisms, 10(10): 1958. DOI: https://www.doi.org/10.3390/microorganisms10101958
Hardiati A, Safika S, Wibawan IWT, Indrawati A, and Pasaribu FH (2021). Isolation and detection of antibiotics resistance genes of *Escherichia coli* from broiler farms in Sukabumi, Indonesia. Journal of Advanced Veterinary and Animal Research, 8(1): 84-90. DOI: https://www.doi.org/10.5455/javar.2021.h489
Kurnia RS, Tarigan S, Nugroho CMH, Silaen OSM, Natalia L, Ibrahim F, and Sudarmono PP (2022). Potency of bacterial sialidase Clostridium perfringens as antiviral of Newcastle disease infections using embryonated chicken egg *in ovo* model. Veterinary World, 15(8): 1896-1905. DOI: https://www.doi.org/10.14202/vetworld.2022.1896-1905
Lacey JA, Stanley D, Keyburn AL, Ford M, Chen H, Johanesen P, Lyras D, and Moore RJ (2018). Clostridium perfringens-mediated necrotic enteritis is not influenced by the pre-existing microbiota but is promoted by large changes in the post-challenge microbiota. Veterinary Microbiology, 227: 119-126. DOI: https://www.doi.org/10.1016/j.vetmic.2018.10.022
Lee KW, Lillehoj HS, Kim W, Park I, Li C, Lu M, and Hofacre CL (2021). Research note: First report on the detection of necrotic enteritis (NE) B-like toxin in biological samples from NE-afflicted chickens using capture enzyme-linked immunosorbent assay. Poultry Science, 100(7): 101190. DOI: https://www.doi.org/10.1016/j.psj.2021.101190
Lee KW, Kim WH, Li C, and Lillehoj HS (2020). Detection of necrotic enteritis B-like toxin secreted by clostridium perfringens using capture enzyme-linked immunosorbent assay. Avian Disease, 64(4): 490-495. DOI: https://www.doi.org/10.1637/0005-2086-64.4.490
Moore RJ (2016). Necrotic enteritis predisposing factors in broiler chickens. Avian Pathology, 45(3): 275-281. DOI: https://www.doi.org/10.1080/03079457.2016.1150587
Nnachi RC, Sui N, Ke B, Luo Z, Bhalla N, He D, and Yang Z (2022). Biosensors for rapid detection of bacterial pathogens in water, food and environment. Environment International, 166: 107357. DOI: https://www.doi.org/10.1016/j.envint.2022.107357
O'Seaghdha M, van Schooten CJ, Kerrigan SW, Emsley J, Silverman GJ, Cox D, Lenting PJ, and Foster TJ (2006). *Staphylococcus aureus* protein-A binding to von Willebrand factor A1 domain is mediated by conserved IgG binding regions. The FEBS Journal, 273(21): 4831-4841. DOI: https://www.doi.org/10.1111/j.1742-4658.2006.05482.x
Ochi S, Oda M, Matsuda H, Ikari S, and Sakurai J (2004). Clostridium perfringens alpha-toxin activates the sphingomyelin metabolism system in sheep erythrocytes. The Journal of Biological Chemistry, 279(13): 12181-12189. DOI: https://www.doi.org/10.1074/jbc.M307046200
Pawaiya RS, Gururaj K, Gangwar NK, Singh DD, Kumar R, and Kumar A (2020). The challenges of diagnosis and control of enterotoxaemia caused by clostridium perfringens in small ruminants. Advances in Microbiology, 10(5): 238-273. DOI: https://www.doi.org/10.4236/aim.2020.105019
Rubio MS, Penha FRAC, Almeida AM, Barbosa FO, and Berchieri Jr A (2019). Duplex real-time PCR using sybr green I for quantification and differential diagnosis between *Salmonella enteritidis* and *Salmonella typhimurium*. Brazilian Journal of Poultry Science, 21(1): 1-6. DOI: https://www.doi.org/10.1590/1806-9061-2018-0776
Salem HM, Ismael E, and Shaalan M (2021). Evaluation of the effects of silver nanoparticles against experimentally induced necrotic enteritis in broiler chickens. International Journal of Nanomedicine, 16: 6783-6796. DOI: https://www.doi.org/10.2147/ijn.S319708
Salem HM and Attia MM (2021). Accidental intestinal myiasis caused by *Musca domestica* L. (Diptera: Muscidae) larvae in broiler chickens: A field study. International Journal of Tropical Insect Science, 41(4): 2549-2554. DOI: https://www.doi.org/10.1007/s42690-021-00492-w
Santiani F, Silva ROS, de Oliveira Júnior CA, Withoeft JA, Cristo TG, Costa LS, Gaspar T, and Casagrande RA (2023). Characterization of coccidiosis and evaluation of suggestive cases of subclinical necrotic enteritis in broilers. Pesquisa Veterinária Brasileira, 43: e07090. DOI: https://www.doi.org/10.1590/1678-5150-PVB-7090
Seal BS, Lillehoj HS, Donovan DM, and Gay CG (2013). Alternatives to antibiotics: A symposium on the challenges and solutions for animal production. Animal Health Research Reviews, 14(1): 78-87. DOI: https://www.doi.org/10.1017/s1466252313000030
Serroni A, Colabella C, Cruciani D, Ciullo M, Crotti S, Papa P, Di Paolo A, Gobbi M, Forti K, Pellegrini M et al. (2022). Identification and characterization of *Clostridium perfringens* atypical CPB2 toxin in cell cultures and field samples using monoclonal antibodies. Toxins, 14(11): 796. Available at: https://www.mdpi.com/2072-6651/14/11/796
Shojadoost B, Vince AR, and Prescott JF (2012). The successful experimental induction of necrotic enteritis in chickens by *Clostridium perfringens*: A critical review. Veterinary Research, 43(1): 74. DOI: https://www.doi.org/10.1186/1297-9716-43-74
Smyth JA (2016). Pathology and diagnosis of necrotic enteritis: Is it clear-cut?. Avian Pathology, 45(3): 282-287. DOI: https://www.doi.org/10.1080/03079457.2016.1158780
Vargas M, Segura A, Herrera M, Villalta M, Angulo Y, Gutiérrez JM, León G, and Burnouf T (2012). Purification of IgG and albumin from human plasma by aqueous two phase system fractionation. Biotechnology Progress, 28(4): 1005-1011. DOI: https://www.doi.org/10.1002/btpr.1565
Wade B and Keyburn A (2015). The true cost of necrotic enteritis. Poultry World, 31(7): 16-17. Available at: https://www.cabidigitallibrary.org/doi/full/10.5555/20153340014
Zahoor I, Ghayas A, and Basheer A (2018). Genetics and genomics of susceptibility and immune response to necrotic enteritis in chicken: A review. Molecular Biology Reports, 45(1): 31-37. DOI: https://www.doi.org/10.1007/s11033-017-4138-8

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